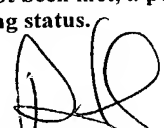


FORM PTO-1390 (REV 11-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 53-99A	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <b>10/030729</b>	
INTERNATIONAL APPLICATION NO. PCT/AU/00799 <i>PCT-AU00-00799</i>		INTERNATIONAL FILING DATE 30 June 2000 (30.06.00)		PRIORITY DATE CLAIMED 1 July 1999 (01.07.99)	
TITLE OF INVENTION <b>GENETIC SEQUENCES ENCODING STEROID AND JUVENILE HORMONE RECEPTOR POLYPEPTIDES AND USES THEREFOR</b>					
APPLICANT(S) FOR DO/EO/US <b>Ronald Johnston HILL, Garry Noel HANNAN</b>					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19<sup>th</sup> month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International application as filed (35 U.S.C. 371(c)(2))           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)):           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>					
<b>Items 11. To 16. below concern document(s) or information included:</b>					
<ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.           <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment         </li> <li>14. <input type="checkbox"/> A substitute specification.</li> <li>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>16. <input type="checkbox"/> Other items or information:</li> </ol>					

U.S. APPLICATION NO. <b>10/030729</b> <small>(known, see 37 CFR 1.6)</small>		INTERNATIONAL APPLICATION NO. PCT/AU00/00799		ATTORNEY'S DOCKET NUMBER 53-99A	
17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO <span style="float: right;"><b>\$1040.00</b></span>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO <span style="float: right;"><b>\$890.00</b></span>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO <span style="float: right;"><b>\$740.00</b></span>  International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) <span style="float: right;"><b>\$710.00</b></span>  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) <span style="float: right;"><b>\$100.00</b></span>  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS PTO USE ONLY</b>	
				\$ 1,040.00	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	99-20 =	79	X \$18.00	\$ 1,422	
Independent claims	17-3 =	14	X \$84.00	\$ 1,176	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+\$280.00	\$ 280	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$ n/a	
<b>SUBTOTAL =</b>				\$	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
<b>TOTAL NATIONAL FEE =</b>				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property				\$	
<b>TOTAL FEES ENCLOSED =</b>				\$ 4,048	
				<b>Amount to be:</b>	\$
				<b>refunded</b>	
				<b>charged</b>	\$
a. <input checked="" type="checkbox"/> A check in the amount of <b>\$ 4,048.00</b> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <b>07-1969</b> in the amount of \$_____ to cover the above fees. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <b>07-1969</b> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO:  GREENLEE, WINNER and SULLIVAN, P.C. 5370 Manhattan Circle, Suite 201 Boulder, CO 80303 Phone: 303-499-8080 Fax: 303-499-8089					
				 SIGNATURE	
				Name: Donna M. Ferber Registration No.: 33,878	

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :  
Hill and Hannan : Group Art Unit: Not yet assigned  
Serial No: Not yet assigned : Examiner: Not yet assigned  
Filed: January 2, 2002

For: GENETIC SEQUENCES ENCODING STEROID AND JUVENILE  
HORMONE RECEPTOR POLYPEPTIDES AND INSECTICIDAL MODALITIES  
THEREFOR (as amended)

CERTIFICATE OF MAILING	
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as EXPRESS MAIL in an envelope addressed to the U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202.	
Date <u>2 January 2002</u>	<u>B. Kroge</u> B. Kroge
Express Mail Receipt No: 881 525 674 US	

## PRELIMINARY AMENDMENT

Commissioner for Patents  
Box PCT  
Washington, DC 20321

Sir:

Please enter the following amendments:

In the title:

**Rewrite the title as follows:**

Genetic Sequences Encoding Steroid and Juvenile Hormone Receptor Polypeptides and  
Uses Therefor.

In the claims:

**Rewrite claims 23, 29, 30-34, 40, 44, 55, 59, 60, 61 and 70-74 as follows:**

23. (Once amended) A gene construct comprising the isolated nucleic acid molecule according to claim 1 operably linked to a promoter sequence.
29. (Once amended) The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor EcR polypeptide of SEQ ID NO:14.
30. (Once amended) The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor EcR polypeptide comprising the amino acid sequence of SEQ ID NO: 42 or encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO:41.
31. (Once amended) The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region is the ligand binding region of the *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
32. (Once amended) The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) comprising the amino acid sequence set forth in SEQ ID NO:40.
33. (Once amended) The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 16.
34. (Once amended) The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region is the ligand binding region fo the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 18.



40. (Once amended) A cell comprising the isolated nucleic acid molecule according to claim 23.
44. (Once amended) A cell that expresses the isolated or recombinant polypeptide according to claim 25.
55. (Once amended) A method of identifying a potential insecticidal compound comprising:
  - (i) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to claim 25 to a steroid response element (SRE) to which said polypeptide binds, in the presence of a candidate compound;
  - (ii) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to claim 25 to a steroid response element (SRE) to which said polypeptide binds, in the absence of said candidate compound; and
  - (iii) comparing the binding assayed at (i) and (ii), wherein a difference in the level of binding indicates that the candidate compound possesses potential insecticidal activity.
59. (Once amended) The method according to claim 56, wherein the reporter gene is the CAT gene or the  $\beta$ -galactosidase gene.
60. (Once amended) The method according to claim 55, wherein the potential insecticidal compound is an insect steroid receptor antagonist or insect juvenile hormone receptor antagonist.
61. (Once amended) The method according to claim 55, wherein the potential insecticidal compound is an insect steroid receptor agonist or insect juvenile hormone receptor agonist.
70. (Once amended) A synthetic compound that interacts with the three dimensional structure of the isolated or recombinant polypeptide according to claim 25 wherein said compound

is capable of binding to said polypeptide or protein to agonize or antagonize the binding activity or bioactivity thereof.

71. (Once amended) A method of identifying a synthetic compound having insecticidal activity comprising contacting the recombinant or isolated polypeptide according to claim 25 with said compound for a time and under conditions sufficient for binding to occur and detecting said binding using a detection means, wherein the occurrence of binding is indicative of potential insecticidal activity of the compound.
72. (Once amended) A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said hormone-binding complex comprises:
  - (i) the ligand-binding region of an ecdysteroid receptor partner protein (USP polypeptide) according to claim 25; and
  - (ii) the EcR polypeptide of an insect ecdysteroid receptor or the ligand binding region thereof.
73. (Once amended) A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said hormone-binding complex comprises:
  - (i) the ligand-binding region of an EcR polypeptide according to claim 25; and
  - (ii) the ecdysteroid receptor partner protein (USP polypeptide) of an insect ecdysteroid receptor or the ligand binding region thereof.
74. (Once amended) A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said complex comprises:

- (i) the ligand binding region of an EcR polypeptide according to claim 25; and
- (ii) the ligand binding region of an ecdysteroid receptor partner protein (USP polypeptide) according to claim 25.

#### REMARKS

The title has been amended to delete the initial word "Novel."

Claims 23, 29, 30-34, 40, 44, 55, 59, 60, 61 and 70-74 have amended to remove multiple dependency. None of the amendments made herein constitutes the addition of new matter.

It is believed that this amendment does not necessitate the payment of any (additional) fees under 37 C.F.R. 1.16-1.17. If the amount submitted is incorrect, please deduct from Deposit Account No. 07-1969 the appropriate fee for this submission and any extension of time required.

Respectfully submitted,



Donna M. Ferber  
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.  
5370 Manhattan Circle, Suite 201  
Boulder, CO 80303  
Telephone (303) 499-8080  
Facsimile: (303) 499-8089  
Email: [winner@greenwin.com](mailto:winner@greenwin.com)

Attorney docket No. 53-99A  
bmk: January 2, 2002

**Marked up version of amended paragraph(s) and claim(s) in the attached  
Preliminary Amendment.**

Docket No.: 53-99A  
Filed January 2, 2002

In the title:

[Novel] Genetic Sequences Encoding Steroid and Juvenile Hormone Receptor Polypeptides  
and Uses Therefor.

In the claims:

23. (Once amended) A gene construct comprising the isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 16] operably linked to a promoter sequence.
29. (Once amended) The isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 28] wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor EcR polypeptide of SEQ ID NO:14.
30. (Once amended) The isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 28] wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor EcR polypeptide comprising the amino acid sequence of SEQ ID NO: 42 or encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO:41.
31. (Once amended) The isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 28] wherein the ligand binding region is the ligand binding region of the *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
32. (Once amended) The isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 28] wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) comprising the amino acid sequence set forth in SEQ ID NO:40.
33. (Once amended) The isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 28] wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 16.

34. (Once amended) The isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 28] wherein the ligand binding region is the ligand binding region fo the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 18.
40. (Once amended) A cell comprising the isolated nucleic acid molecule according to [anyh one of claims 1 to 16 or the gene construct according to] claim 23 [or 24].
44. (Once amended) A cell that expresses the isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 39].
55. (Once amended) A method of identifying a potential insecticidal compound comprising:
  - (i) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to [any one of] claim[s] 25 [to 39] to a steroid response element (SRE) to which said polypeptide binds, in the presence of a candidate compound;
  - (ii) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to [any one of] claim[s] 25 [to 39] to a steroid response element (SRE) to which said polypeptide binds, in the absence of said candidate compound; and
  - (iii) comparing the binding assayed at (i) and (ii), wherein a difference in the level of binding indicates that the candidate compound possesses potential insecticidal activity.
59. (Once amended) The method according to [any one of] claim[s] 56 [to 58], wherein the reporter gene is the CAT gene or the  $\beta$ -galactosidase gene.
60. (Once amended) The method according to [any one of] claim[s] 55 [to 59], wherein the potential insecticidal compound is an insect steroid receptor antagonist or insect juvenile hormone receptor antagonist.
61. (Once amended) The method according to [any one of] claim[s] 55 [to 59], wherein the potential insecticidal compound is an insect steroid receptor agonist or insect juvenile hormone receptor agonist.
70. (Once amended) A synthetic compound that interacts with the three dimensional structure of the isolated or recombinant polypeptide according to [any one of] claim[s] 25 [to 39] wherein said compound is capable of binding to said polypeptide or protein to agonize or antagonize the binding activity or bioactivity thereof.

71. (Once amended) A method of identifying a synthetic compound having insecticidal activity comprising contacting the recombinant or isolated polypeptide according to [any one of] claim[s] 25 [to 39] with said compound for a time and under conditions sufficient for binding to occur and detecting said binding using a detection means, wherein the occurrence of binding is indicative of potential insecticidal activity of the compound.
72. (Once amended) A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said hormone-binding complex comprises:
  - (i) the ligand-binding region of an ecdysteroid receptor partner protein (USP polypeptide) according to [any one of] claim[s] 25 [to 28 or any one of claims 31 to 35 or claim 37 or 38]; and
  - (ii) the EcR polypeptide of an insect ecdysteroid receptor or the ligand binding region thereof.
73. (Once amended) A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said hormone-binding complex comprises:
  - (i) the ligand-binding region of an EcR polypeptide according to [any one of] claim[s] 25 [to 30 or claim 36 or 39]; and
  - (ii) the ecdysteroid receptor partner protein (USP polypeptide) of an insect ecdysteroid receptor or the ligand binding region thereof.
74. (Once amended) A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said complex comprises:
  - (i) the ligand binding region of an EcR polypeptide according to [any one of] claim[s] 25 [to 30 or claim 36 or 39]; and
  - (ii) the ligand binding region of an ecdysteroid receptor partner protein (USP polypeptide) according to [any one of] claim[s] 25 [to 28 or any one of claims 31 to 35 or claim 37 or 38].

13 REC'D PCT/PTO 23 JUN 2002  
10/030729

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :  
Hill and Hannan : Group Art Unit: Not yet assigned  
Serial No: Not yet assigned : Examiner: Not yet assigned  
371 Filing date: January 2, 2002 : Confirmation No. 8254  
International Filing Date: June 30, 2000

For: GENETIC SEQUENCES ENCODING STEROID AND JUVENILE  
HORMONE RECEPTOR POLYPEPTIDES AND INSECTICIDAL MODALITIES  
THEREFOR (as amended)

CERTIFICATE OF MAILING	
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as EXPRESS MAIL in an envelope addressed to the U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202.	
<i>20 June 2002</i> Date	<i>B. Kroge</i> B. Kroge
Express Mail Receipt No: EL 946 696 193	

SECOND PRELIMINARY AMENDMENT

Commissioner for Patents  
Box PCT  
Washington, DC 20321

Sir:

Please enter the following amendments:

In the claims:

Rewrite claims 5-10, 22, 47, 50 and 53





22. (Once amended) The method according to claim 17 further comprising isolating the identified nucleic acid molecule.
47. (Once amended) The method of identifying a modulator of steroid receptor-mediated gene expression or juvenile hormone receptor-mediated gene expression comprising:
  - (i) assaying the expression of a reporter gene in the presence of the recombinant or isolated polypeptide according to any one of claims 25 or 35 to 39 and a potential modulator;
  - (ii) assaying the expression of the reporter gene in the presence of the recom binant or isolated polypeptide according to any one of claims 25 or 35 to 39 and without said potential modulator; and
  - (iii) comparing expression of the reporter gene at (i) and (ii),wherein expression of said reporter gene is effected by the binding of said polypeptide to a steroid response element (SRE) or a promoter sequence comprising said SRE, and wherein a different level of expression at (iii) indicates that said potential modulator is a modulator of steroid receptor-mediated gene expression.
50. (Once amended) The method according to claim 47 wherein the reporter gene is the CAT gene or the  $\beta$ -galactosidase gene.
53. (Once amended) The method of claim 47, wherein the modulator is an agonist or antagonist and wherein said modulator is a synthetic chemical that mimics the structure of a ligand of said receptor, thereby modulating binding of said ligand to said receptor.

# REMARKS

Claims 22, 47, 50 and 53 have amended to remove some of the multiple dependency. None of the amendments made herein constitutes the addition of new matter.

This amendment is accompanied by a Petition for Extension of Time (one month) and a check in the amount of \$110.00 as required under 37 C.F.R. 1.17. It is believed that this amendment does not necessitate the payment of any additional fees under 37 C.F.R. 1.16-1.17. If the amount submitted is incorrect, however, please deduct from Deposit Account No. 07-1969 the appropriate fee for this submission and any extension of time required.

Respectfully submitted,



Donna M. Ferber  
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.  
5370 Manhattan Circle, Suite 201  
Boulder, CO 80303  
Telephone (303) 499-8080  
Facsimile: (303) 499-8089  
Email: [winner@greenwin.com](mailto:winner@greenwin.com)

Attorney docket No. 53-99A  
bmk: June 20, 2002

**Marked up version of amended claim(s) in the attached  
Second Preliminary Amendment.**

371 Filing date: January 2, 2002  
International Filing Date: June 30, 2000

5. (Once amended) The isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 4] wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor EcR polypeptide of SEQ ID NO:14.
6. (Once amended) The isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 4] wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor EcR polypeptide, said EcR polypeptide comprising the amino acid sequence of SEQ ID NO:42 or encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO:41.
7. (Once amended) The isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 4] wherein the ligand binding region is the ligand binding region of the *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8.
8. (Once amended) The isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 4] wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) comprising the amino acid sequence set forth in SEQ ID NO:40.
9. (Once amended) The isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 4] wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO:16.
10. (Once amended) The isolated nucleic acid molecule according to [any one of] claim[s] 1 [to 4] wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO:18.
22. (Once amended) The method according to [any one of] claim[s] 17 [to 21] further comprising isolating the identified nucleic acid molecule.
47. (Once amended) [A] The method of identifying a modulator of steroid receptor-mediated gene expression or juvenile hormone receptor-mediated gene expression comprising:
  - (i) assaying the expression of a reporter gene in the presence of the recombinant or isolated polypeptide according to any one of claims 25 or 35 to 39 and a potential modulator;

(ii) assaying the expression of the reporter gene in the presence of the recombinant or isolated polypeptide according to any one of claims 25 or 35 to 39 and without said potential modulator; and

(iii) comparing expression of the reporter gene at (i) and (ii),

wherein expression of said reporter gene is effected by the binding of said polypeptide to a steroid response element (SRE) or a promoter sequence comprising said SRE, and wherein a different level of expression at (iii) indicates that said potential modulator is a modulator of steroid receptor-mediated gene expression.

50. (Once amended) The method according to [any one of] claim[s] 47 [to 49], wherein the reporter gene is the CAT gene or the  $\beta$ -galactosidase gene.

53. (Once amended) The method of claim [51 or 52] 47, wherein the modulator is an agonist or antagonist and wherein said modulator is a synthetic chemical that mimics the structure of a ligand of said receptor, thereby modulating binding of said ligand to said receptor.

13 Recd PCT/PTO 20 JUN 2002  
10/030729

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :  
Hill and Hannan : Group Art Unit: Not yet assigned  
Serial No: Not yet assigned : Examiner: Not yet assigned  
371 Filing date: January 2, 2002 : Confirmation No. 8254  
International Filing Date: June 30, 2000

STATEMENT UNDER 37 C.F.R. §1.821-824

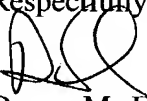
Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements mailed March 20, 2002,  
Applicants submit a write-protected diskette copy of the Sequence Listing in computer-readable  
form as required by 37 C.F.R. §1.821(e), and on paper as pages 1 - 51.

In compliance with 37 C.F.R. §1.821(f), the undersigned states that the content of the  
paper copies and computer-readable copies of the Sequence Listing are the same.

Respectfully submitted,

  
Donna M. Ferber  
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.  
5370 Manhattan Circle, Suite 201  
Boulder, CO 80303  
Telephone (303) 499-8080  
Facsimile: (303) 499-8089  
Email: winner@greenwin.com

Attorney Docket No.: 53-99A  
bmk: June 20, 2002

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited  
with the United States Postal Service with sufficient  
postage as EXPRESS MAIL in an envelope addressed to  
the Commissioner for Patents, Washington, D.C., 20231

20 June 2002 B. Kroge  
Date B. Kroge  
Express Mail Receipt No. EL 946 696 193 US

10/030729

## SEQUENCE LISTING

<110> Hill, Ronald J.  
Hannan, Garry N.

<120> GENETIC SEQUENCES ENCODING STEROID AND JUVENILE HORMONE  
RECEPTOR POLYPEPTIDES AND INSECTICIDAL MODALITIES  
THEREFOR

<130> 53-99A

<140> 10/030,729

<141> 2002-01-02

<150> PCT/AU00/00799

<151> 2000-06-30

<150> US 09/346,470

<151> 1999-07-01

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<170> PatentIn Ver. 2.0

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Pro	Gln	Glu	Ile	Lys	Pro	Asp	Ile	Ser	Leu	Leu	Asn	Glu	Asn	Asn	Thr
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Ser	Ser	Tyr	Ser	Pro	Lys	Pro	Gly	Ser	Pro	Asn	Pro	Phe	Ala	Ile	Gly
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Leu	Gln	Ala	Ile	Asn	Ala	Val	Ala	Ala	Ala	Asn	Ala	Asn	Asn	Gln	Asn
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Gln	Met	Leu	Gln	Thr	Thr	Pro	Pro	Gln	Gln	Gln	Gln	Tyr	Pro	Pro	Asn
65					70					75					80
His	Pro	Leu	Ser	Gly	Ser	Lys	His	Leu	Cys	Ser	Ile	Cys	Gly	Asp	Arg
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Ala	Ser	Gly	Lys	His	Tyr	Gly	Val	Tyr	Ser	Cys	Glu	Gly	Cys	Lys	Gly
			100					105					110		
Phe	Phe	Lys	Arg	Thr	Val	Arg	Lys	Asp	Leu	Thr	Tyr	Ala	Cys	Arg	Glu
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Asp	Arg	Asn	Cys	Ile	Ile	Asp	Lys	Arg	Gln	Arg	Asn	Arg	Cys	Gln	Tyr
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Cys	Arg	Tyr	Gln	Lys	Cys	Leu	Ala	Cys	Gly	Met	Lys	Arg	Glu	Ala	Val
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Gln	Glu	Glu	Arg	Gln	Arg	Gly	Thr	Arg	Ala	Ala	Asn	Ala	Arg	Ala	Ala
				165					170					175	
Gly	Ala	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Val	Ser	Asn	Val	Val
			180					185					190		
Gly	Ala	Gly	Gly	Glu	Asp	Phe	Lys	Pro	Ser	Ser	Ser	Leu	Arg	Asp	Leu
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Thr	Ile	Glu	Arg	Ile	Ile	Glu	Ala	Glu	Gln	Lys	Ala	Glu	Ser	Leu	Ser
	210					215					220				

Gly Asp Asn Val Leu Pro Phe Leu Arg Val Gly Asn Asn Ser Met Val  
 225 230 235 240

Gln His Asp Tyr Lys Gly Ala Val Ser His Leu Cys Gln Met Val Asn  
 245 250 255

Lys Gln Leu Tyr Gln Met Val Glu Tyr Ala Arg Arg Thr Pro His Phe  
 260 265 270

Thr His Leu Gln Arg Glu Asp Gln Ile Leu Leu Leu Lys Ala Gly Trp  
 275 280 285

Asn Glu Leu Leu Ile Ala Asn Val Ala Trp Cys Ser Ile Glu Ser Leu  
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Asp Ala Glu Tyr Ala Ser Pro Gly Thr Val His Asp Gly Ser Phe Gly  
 305 310 315 320

Arg Arg Ser Pro Val Arg Gln Pro Gln Gln Leu Phe Leu Asn Gln Asn  
 325 330 335

Phe Ser Tyr His Arg Asn Ser Ala Ile Lys Ala Asn Val Val Ser Ile  
 340 345 350

Phe Asp Arg Ile Leu Ser Glu Leu Ser Ile Lys Met Lys Arg Leu Asn  
 355 360 365

Ile Asp Arg Ser Glu Leu Ser Cys Leu Lys Ala Ile Ile Leu Phe Asn  
 370 375 380

Pro Asp Ile Arg Gly Leu Lys Cys Arg Ala Asp Val Glu Val Cys Arg  
 385 390 395 400

Glu Lys Ile Tyr Ala Cys Leu Asp Glu His Cys Arg Thr Glu His Pro  
 405 410 415

Gly Asp Asp Gly Arg Phe Ala Gln Leu Leu Leu Arg Leu Pro Ala Leu  
 420 425 430

Arg Ser Ile Ser Leu Lys Cys Leu Asp His Leu Phe Phe Phe Arg Leu  
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Pro Ile Cys  
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<210> 7  
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Arg Ala Ala Gly Ala Gly Gly Gly Gly Gly Gly Gly Gly Val Ser	
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aat gtg gtt ggt gct ggc gga gaa gac ttt aaa ccc agc agt tca tta	747
Asn Val Val Gly Ala Gly Gly Glu Asp Phe Lys Pro Ser Ser Ser Leu	
190 195 200 205	
cgt gat ctc act ata gaa cgc atc att gaa gcc gag caa aag gct gaa	795
Arg Asp Leu Thr Ile Glu Arg Ile Ile Glu Ala Glu Gln Lys Ala Glu	
210 215 220	
tct ttg agc ggt gat aac gtg ttg ccc ttt ttg cgc gtt ggc aac aat	843
Ser Leu Ser Gly Asp Asn Val Leu Pro Phe Leu Arg Val Gly Asn Asn	
225 230 235	
tcc atg gta caa cac gac tac aaa ggc gcg gta tct cat ctc tgc cag	891
Ser Met Val Gln His Asp Tyr Lys Gly Ala Val Ser His Leu Cys Gln	
240 245 250	
atg gtt aac aaa caa ctc tac caa atg gtt gaa tat gca cgt cga aca	939
Met Val Asn Lys Gln Leu Tyr Gln Met Val Glu Tyr Ala Arg Arg Thr	
255 260 265	
cca cat ttt aca cat ttg cag cgt gag gat cag ata cta ttg tta aag	987
Pro His Phe Thr His Leu Gln Arg Glu Asp Gln Ile Leu Leu Leu Lys	
270 275 280 285	
gct ggc tgg aat gaa ctg cta att gca aat gtt gcc tgg tgc agt att	1035
Ala Gly Trp Asn Glu Leu Leu Ile Ala Asn Val Ala Trp Cys Ser Ile	
290 295 300	
gag tct ctg gat gcc gaa tat gcc tct cct ggt acg gta cat gac ggt	1083
Glu Ser Leu Asp Ala Glu Tyr Ala Ser Pro Gly Thr Val His Asp Gly	
305 310 315	
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Ser Phe Gly Arg Arg Ser Pro Val Arg Gln Pro Gln Gln Leu Phe Leu	
320 325 330	
aat cag aat ttc tcg tat cat cgc aat agt gct att aag gcc aat gtt	1179
Asn Gln Asn Phe Ser Tyr His Arg Asn Ser Ala Ile Lys Ala Asn Val	
335 340 345	
gtt tca att ttc gat cgt atc ctc tcg gag ttg agc atc aaa atg aaa	1227
Val Ser Ile Phe Asp Arg Ile Leu Ser Glu Leu Ser Ile Lys Met Lys	
350 355 360 365	



cgt ctt aac atc gat cgc tcg gag ttg tcg tgt ctg aag gca atc ata 1275  
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ctc ttc aat cca gac ata cgc ggt ctg aaa tgt cga gcc gac gtc gag 1323  
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gta tgt cgt gaa aaa atc tat gcc tgt ctg gac gaa cac tgc cgc aca 1371  
 Val Cys Arg Glu Lys Ile Tyr Ala Cys Leu Asp Glu His Cys Arg Thr  
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gaa cat cca ggt gat gat ggc cgc ttt gct cag cta cta cta agg ttg 1419  
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ccc gca ttg cgt tcc atc agt ctc aaa tgt ctc gat cat ttg ttt ttc 1467  
 Pro Ala Leu Arg Ser Ile Ser Leu Lys Cys Leu Asp His Leu Phe Phe  
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ttc cgt tta ata ggc gaa aga gca ttg gag gaa tta att gct gag caa 1515  
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<212> PRT

<213> Lucilia cuprina

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Ser Ser Tyr Ser Pro Lys Pro Gly Ser Pro Asn Pro Phe Ala Ile Gly  
 35 40 45

Leu Gln Ala Ile Asn Ala Val Ala Ala Ala Asn Ala Asn Asn Gln Asn  
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Gln Met Leu Gln Thr Thr Pro Pro Gln Gln Gln Gln Tyr Pro Pro Asn  
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His Pro Leu Ser Gly Ser Lys His Leu Cys Ser Ile Cys Gly Asp Arg  
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Ala	Ser	Gly	Lys	His	Tyr	Gly	Val	Tyr	Ser	Cys	Glu	Gly	Cys	Lys	Gly	
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Phe	Phe	Lys	Arg	Thr	Val	Arg	Lys	Asp	Leu	Thr	Tyr	Ala	Cys	Arg	Glu	
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Asp	Arg	Asn	Cys	Ile	Ile	Asp	Lys	Arg	Gln	Arg	Asn	Arg	Cys	Gln	Tyr	
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Cys	Arg	Tyr	Gln	Lys	Cys	Leu	Ala	Cys	Gly	Met	Lys	Arg	Glu	Ala	Val	
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Gln	Glu	Glu	Arg	Gln	Arg	Gly	Thr	Arg	Ala	Ala	Asn	Ala	Arg	Ala	Ala	
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Gly	Ala	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Val	Ser	Asn	Val	Val	
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Gly	Ala	Gly	Gly	Glu	Asp	Phe	Lys	Pro	Ser	Ser	Ser	Leu	Arg	Asp	Leu	
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Thr	Ile	Glu	Arg	Ile	Ile	Glu	Ala	Glu	Gln	Lys	Ala	Glu	Ser	Leu	Ser	
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Gly	Asp	Asn	Val	Leu	Pro	Phe	Leu	Arg	Val	Gly	Asn	Asn	Ser	Met	Val	
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Gln	His	Asp	Tyr	Lys	Gly	Ala	Val	Ser	His	Leu	Cys	Gln	Met	Val	Asn	
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Lys	Gln	Leu	Tyr	Gln	Met	Val	Glu	Tyr	Ala	Arg	Arg	Thr	Pro	His	Phe	
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Asp	Ala	Glu	Tyr	Ala	Ser	Pro	Gly	Thr	Val	His	Asp	Gly	Ser	Phe	Gly	
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Arg	Arg	Ser	Pro	Val	Arg	Gln	Pro	Gln	Gln	Leu	Phe	Leu	Asn	Gln	Asn	
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Phe	Ser	Tyr	His	Arg	Asn	Ser	Ala	Ile	Lys	Ala	Asn	Val	Val	Ser	Ile	
			340						345			350				
Phe	Asp	Arg	Ile	Leu	Ser	Glu	Leu	Ser	Ile	Lys	Met	Lys	Arg	Leu	Asn	
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Tyr Gln Cys Lys Tyr Gly Asn Asn Cys Glu Ile Asp Met Tyr Met Arg  
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cgg aag tgc cag gag tgc cgg ctg aaa aaa tgc ctg acc gtc ggc atg 336  
Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Thr Val Gly Met  
100 105 110

agg cct gaa tgt gtt gta cct gaa gtt caa tgc gca gta aaa aga aag 384  
Arg Pro Glu Cys Val Val Pro Glu Val Gln Cys Ala Val Lys Arg Lys  
115 120 125

gag aaa aaa gct caa cga gaa aaa gat aaa cca aat tct act aca gac 432  
Glu Lys Lys Ala Gln Arg Glu Lys Asp Lys Pro Asn Ser Thr Thr Asp  
130 135 140

att tct cct gaa ata ata aaa ata gaa cct aca gag atg aag att gaa 480  
Ile Ser Pro Glu Ile Ile Lys Ile Glu Pro Thr Glu Met Lys Ile Glu  
145 150 155 160

tgt ggt gaa cca atg ata atg ggc aca cct atg ccg act gta cct tac 528  
Cys Gly Glu Pro Met Ile Met Gly Thr Pro Met Pro Thr Val Pro Tyr  
165 170 175

gtg aaa cct ttg agt tct ctc gtg ccg aat tcg gca cga gtc acg ggt 576  
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Tyr Lys Phe  
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Arg Phe Leu Phe Ile Ser Glu Gln Pro Pro Pro Glu Glu Leu Cys Leu  
35 40 45

Val Cys Gly Asp Arg Ser Ser Gly Tyr His Tyr Asn Ala Leu Thr Cys  
50 55 60

Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Ile Thr Lys Asn Ala Val  
65 70 75 80

Tyr Gln Cys Lys Tyr Gly Asn Asn Cys Glu Ile Asp Met Tyr Met Arg  
85 90 95

Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Thr Val Gly Met  
100 105 110

Arg Pro Glu Cys Val Val Pro Glu Val Gln Cys Ala Val Lys Arg Lys  
115 120 125

Glu Lys Lys Ala Gln Arg Glu Lys Asp Lys Pro Asn Ser Thr Thr Asp  
130 135 140

Ile Ser Pro Glu Ile Ile Lys Ile Glu Pro Thr Glu Met Lys Ile Glu  
145 150 155 160

Cys Gly Glu Pro Met Ile Met Gly Thr Pro Met Pro Thr Val Pro Tyr  
165 170 175

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caaacgtata atgagttcac ccgatgaaaa tgaaagtcaa cacgatgcat catttcgtca 360  
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gcc gcc ggt atc ggt ggc ggc ggt gtc ggc ggc ctc atg tcg tac aac 96  
Ala Ala Gly Ile Gly Gly Gly Gly Val Gly Gly Leu Met Ser Tyr Asn  
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cgt ggc cgt ggc ggc acc gag gtc atc atc aaa ccc cgt agt cct gcc 144  
Arg Gly Arg Gly Gly Thr Glu Val Ile Ile Lys Pro Arg Ser Pro Ala  
35 40 45  
gtg gtg cag gtg gcc acc ggt ggc agt tac cac ggc ctg ccg gcg gcc 192  
Val Val Gln Val Ala Thr Gly Gly Ser Tyr His Gly Leu Pro Ala Ala  
50 55 60  
tcc gac gcc gtc atc gtg cgc agc ccg cca ggc ggc cac ttg ccc ggg 240  
Ser Asp Ala Val Ile Val Arg Ser Pro Pro Gly Gly His Leu Pro Gly  
65 70 75 80  
ccg cag cag caa gtg ccg ccg tcc cgc aac ggc tgt tcc acc ctg ttt 288  
Pro Gln Gln Gln Val Pro Pro Ser Arg Asn Gly Cys Ser Thr Leu Phe  
85 90 95  
agc gac atc gct ggc gtc aag cga ctc agg ccc gac gat tgg ttg gcc 336  
Ser Asp Ile Ala Gly Val Lys Arg Leu Arg Pro Asp Asp Trp Leu Ala  
100 105 110

[illegible]

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Thr Asp Ile Ser Pro Glu Ile Ile Lys Ile Glu Pro Thr Glu Met Lys	
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Ile Glu Cys Gly Glu Pro Met Ile Met Gly Thr Pro Met Pro Thr Val	
340 345 350	
cct tac gtg aaa cct ttg agt tct gaa caa aaa gaa ctg atc cac cga	1104
Pro Tyr Val Lys Pro Leu Ser Ser Glu Gln Lys Glu Leu Ile His Arg	
355 360 365	
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Leu Val Tyr Phe Gln Asp Gln Tyr Glu Ala Pro Ser Glu Lys Asp Met	
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Lys Arg Leu Thr Ile Asn Asn Gln Asn Met Asp Glu Tyr Asp Glu Glu	
385 390 395 400	
aaa caa agt gac acc aca tat cga atc atc act gag atg aca ata ctc	1248
Lys Gln Ser Asp Thr Thr Tyr Arg Ile Ile Thr Glu Met Thr Ile Leu	
405 410 415	
aca gtt caa ctg att gtt gag ttt gcc aaa cga tta cca ggt ttc gat	1296
Thr Val Gln Leu Ile Val Glu Phe Ala Lys Arg Leu Pro Gly Phe Asp	
420 425 430	
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Lys Leu Val Arg Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser	
435 440 445	
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Glu Ala Met Met Phe Arg Val Ala Arg Lys Tyr Asp Ile Thr Thr Asp	
450 455 460	
tca ata gtg ttt gct aac aac cag cca ttt tca gct gat tca tat aac	1440
Ser Ile Val Phe Ala Asn Asn Gln Pro Phe Ser Ala Asp Ser Tyr Asn	
465 470 475 480	
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Lys Ala Gly Leu Gly Asp Ala Ile Glu Asn Gln Leu Ser Phe Ser Arg	
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Phe Met Tyr Asn Met Lys Val Asp Asn Ala Glu Tyr Ala Leu Leu Thr	
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gcc atc gtc ata ttt tcg agt agg cca aat tta cta gat ggt tgg aaa	1584
Ala Ile Val Ile Phe Ser Ser Arg Pro Asn Leu Leu Asp Gly Trp Lys	
515 520 525	



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Val Glu Lys Ile Gln Glu Ile Tyr Leu Glu Ser Leu Lys Ala Tyr Val  
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gat aat cga gac cgt gac aca gca act gta cga tat gcg cga ctt ctc 1680  
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545 550 555 560

tca gta ctt aca gaa ttg cgc aca tta ggc aat gaa aac tct gag cta 1728  
Ser Val Leu Thr Glu Leu Arg Thr Leu Gly Asn Glu Asn Ser Glu Leu  
565 570 575

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Cys Met Thr Leu Lys Leu Lys Asn Arg Val Val Pro Pro Phe Leu Ala  
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35 40 45

Val Val Gln Val Ala Thr Gly Gly Ser Tyr His Gly Leu Pro Ala Ala  
50 55 60

Ser Asp Ala Val Ile Val Arg Ser Pro Pro Gly Gly His Leu Pro Gly  
65 70 75 80

Pro Gln Gln Gln Val Pro Pro Ser Arg Asn Gly Cys Ser Thr Leu Phe  
85 90 95

Ser Asp Ile Ala Gly Val Lys Arg Leu Arg Pro Asp Asp Trp Leu Ala  
100 105 110

Val Asn Ser Pro Pro Ala Ser Ser Pro Gly Thr Ser His Ile Ser Tyr  
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Thr	Val	Ile	Ser	Asn	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly
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Tyr	Asn	Thr	Ser	Pro	Met	Ser	Thr	Asn	Ser	Tyr	Asp	Pro	Tyr	Ser	Pro
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Ser	Gly	Val	Ser	Ser	His	Ser	Asp	Gly	Leu	Lys	Lys	Lys	Lys	Leu	Asn
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His	Thr	Pro	Ser	Thr	Gly	Val	Val	Asn	Thr	Ser	Ala	Ser	Gly	Pro	Gly
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210						215				220					
Cys	Leu	Val	Cys	Gly	Asp	Arg	Ser	Ser	Gly	Tyr	His	Tyr	Asn	Ala	Leu
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Thr	Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg	Ser	Ile	Thr	Lys	Asn
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Ile	Glu	Cys	Gly	Glu	Pro	Met	Ile	Met	Gly	Thr	Pro	Met	Pro	Thr	Val
		340						345				350			
Pro	Tyr	Val	Lys	Pro	Leu	Ser	Ser	Glu	Gln	Lys	Glu	Leu	Ile	His	Arg
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Leu	Val	Tyr	Phe	Gln	Asp	Gln	Tyr	Glu	Ala	Pro	Ser	Glu	Lys	Asp	Met
370						375				380					
Lys	Arg	Leu	Thr	Ile	Asn	Asn	Gln	Asn	Met	Asp	Glu	Tyr	Asp	Glu	Glu
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Lys Gln Ser Asp Thr Thr Tyr Arg Ile Ile Thr Glu Met Thr Ile Leu  
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 Thr Val Gln Leu Ile Val Glu Phe Ala Lys Arg Leu Pro Gly Phe Asp  
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 Lys Leu Val Arg Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser  
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 Glu Ala Met Met Phe Arg Val Ala Arg Lys Tyr Asp Ile Thr Thr Asp  
                             450                            455                            460  
 Ser Ile Val Phe Ala Asn Asn Gln Pro Phe Ser Ala Asp Ser Tyr Asn  
                             465                            470                            475                            480  
 Lys Ala Gly Leu Gly Asp Ala Ile Glu Asn Gln Leu Ser Phe Ser Arg  
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 Phe Met Tyr Asn Met Lys Val Asp Asn Ala Glu Tyr Ala Leu Leu Thr  
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 Ala Ile Val Ile Phe Ser Ser Arg Pro Asn Leu Leu Asp Gly Trp Lys  
                             515                            520                            525  
 Val Glu Lys Ile Gln Glu Ile Tyr Leu Glu Ser Leu Lys Ala Tyr Val  
                             530                            535                            540  
 Asp Asn Arg Asp Arg Asp Thr Ala Thr Val Arg Tyr Ala Arg Leu Leu  
                             545                            550                            555                            560  
 Ser Val Leu Thr Glu Leu Arg Thr Leu Gly Asn Glu Asn Ser Glu Leu  
                             565                            570                            575  
 Cys Met Thr Leu Lys Leu Lys Asn Arg Val Val Pro Pro Phe Leu Ala  
                             580                            585                            590  
 Glu Ile Trp Asp Val Met Pro  
                             595

<210> 15  
 <211> 1131  
 <212> DNA  
 <213> Myzus persicae

<220>  
 <221> CDS  
 <222> (1)..(1131)

<400> 15

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Met	Tyr	Ser	Asn	Ser	Tyr	Thr	Met	Tyr	Ser	Ser	Asp	Arg	Leu	Tyr	Ser	
1				5					10					15		
gtc	gat	cgg	aac	agt	atg	atg	aat	aat	tct	tgc	aac	gta	caa	gac	tct	96
Val	Asp	Arg	Asn	Ser	Met	Met	Asn	Asn	Ser	Cys	Asn	Val	Gln	Asp	Ser	
			20					25					30			
ccg	aat	tac	ccg	ccc	aac	cat	cca	ctc	agc	ggg	tcg	aaa	cat	ctg	tgc	144
Pro	Asn	Tyr	Pro	Pro	Asn	His	Pro	Leu	Ser	Gly	Ser	Lys	His	Leu	Cys	
			35				40					45				
tcc	ata	tgc	ggc	gat	cgc	gcc	agt	gga	aaa	cat	tac	gga	gtc	tac	agc	192
Ser	Ile	Cys	Gly	Asp	Arg	Ala	Ser	Gly	Lys	His	Tyr	Gly	Val	Tyr	Ser	
	50					55					60					
tgc	gag	ggg	tgc	aaa	ggg	ttc	ttc	aaa	cgc	aca	gtg	agg	aaa	aat	ttg	240
Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Lys	Arg	Thr	Val	Arg	Lys	Asn	Leu	
65				70					75					80		
tca	tac	gcg	tgt	cgc	gaa	gaa	aac	aaa	tgc	atc	atc	gac	aag	cgc	caa	288
Ser	Tyr	Ala	Cys	Arg	Glu	Glu	Asn	Lys	Cys	Ile	Ile	Asp	Lys	Arg	Gln	
				85					90					95		
cga	aat	cgg	tgc	caa	tac	tgc	agg	tat	caa	aaa	tgt	ttg	acc	atg	ggc	336
Arg	Asn	Arg	Cys	Gln	Tyr	Cys	Arg	Tyr	Gln	Lys	Cys	Leu	Thr	Met	Gly	
			100					105					110			
atg	aaa	aga	gaa	gct	gtg	cag	gaa	gaa	aga	caa	cgt	aca	aaa	gaa	cga	384
Met	Lys	Arg	Glu	Ala	Val	Gln	Glu	Glu	Arg	Gln	Arg	Thr	Lys	Glu	Arg	
			115				120					125				
gat	cat	aat	aac	atc	gaa	gtt	gaa	ccc	acg	agc	agt	tct	aat	act	gat	432
Asp	His	Asn	Asn	Ile	Glu	Val	Glu	Pro	Thr	Ser	Ser	Ser	Asn	Thr	Asp	
	130					135					140					
atg	cca	gtg	gaa	ctc	ata	tta	agg	gct	gag	aat	aaa	gct	gat	gct	ata	480
Met	Pro	Val	Glu	Leu	Ile	Leu	Arg	Ala	Glu	Asn	Lys	Ala	Asp	Ala	Ile	
145				150						155					160	
aag	act	gaa	caa	cag	tat	ata	gag	caa	cga	cat	cct	caa	cat	act	gtt	528
Lys	Thr	Glu	Gln	Gln	Tyr	Ile	Glu	Gln	Arg	His	Pro	Gln	His	Thr	Val	
				165					170					175		
ggg	gct	att	tgt	caa	gca	act	gac	aag	cag	tta	ata	caa	ctt	gtt	gaa	576
Gly	Ala	Ile	Cys	Gln	Ala	Thr	Asp	Lys	Gln	Leu	Ile	Gln	Leu	Val	Glu	
			180					185					190			
tgg	gcc	aag	cat	ata	ccg	cat	ttt	aaa	aat	tta	cct	cta	ggc	gat	caa	624
Trp	Ala	Lys	His	Ile	Pro	His	Phe	Lys	Asn	Leu	Pro	Leu	Gly	Asp	Gln	
			195				200					205				

gtt tta tta ttg aga gct ggt tgg aat gag ttg atg att gca gca ttt	672
Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Met Ile Ala Ala Phe	
210 215 220	
tcc cat aga tca atc agt gta aaa gat ggt ata gtc tta gct act gga	720
Ser His Arg Ser Ile Ser Val Lys Asp Gly Ile Val Leu Ala Thr Gly	
225 230 235 240	
ctt act gtt gac aga gat tca gct cac caa gct ggt gtt gaa gct ata	768
Leu Thr Val Asp Arg Asp Ser Ala His Gln Ala Gly Val Glu Ala Ile	
245 250 255	
ttt gat cgt gta ctc act gaa ctc gtt gct aaa atg aga gat atg ggt	816
Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Asp Met Gly	
260 265 270	
atg gat aga aca gag ctt ggc tgt ttg cgt act att att ctt ttt aat	864
Met Asp Arg Thr Glu Leu Gly Cys Leu Arg Thr Ile Ile Leu Phe Asn	
275 280 285	
cca ggt tca aaa ggt ttg cag tct gtg aat gaa gtg caa gta ctg cgt	912
Pro Gly Ser Lys Gly Leu Gln Ser Val Asn Glu Val Gln Val Leu Arg	
290 295 300	
gat aag gtt tat gtt gcg tta gaa gaa tat tgt cgt aca aca cat cca	960
Asp Lys Val Tyr Val Ala Leu Glu Glu Tyr Cys Arg Thr Thr His Pro	
305 310 315 320	
gaa gaa cct gga cga ttt gct aaa cta ctt ctt cgg ctt cct tca tta	1008
Glu Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu	
325 330 335	
cgt tca att gga tta aaa tgt ctg gaa cat tta ttc ttt tat aaa ctt	1056
Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Tyr Lys Leu	
340 345 350	
att ggc gat tcc cca att gat aca ttt tta atg gaa gtt ctc gaa tca	1104
Ile Gly Asp Ser Pro Ile Asp Thr Phe Leu Met Glu Val Leu Glu Ser	
355 360 365	
tct tca cat gac gtt caa gta gct aca	1131
Ser Ser His Asp Val Gln Val Ala Thr	
370 375	

<210> 16  
 <211> 377  
 <212> PRT  
 <213> Myzus persicae  
 <400> 16

Met Tyr Ser Asn Ser Tyr Thr Met Tyr Ser Ser Asp Arg Leu Tyr Ser  
1 5 10 15

Val Asp Arg Asn Ser Met Met Asn Asn Ser Cys Asn Val Gln Asp Ser  
20 25 30

Pro Asn Tyr Pro Pro Asn His Pro Leu Ser Gly Ser Lys His Leu Cys  
35 40 45

Ser Ile Cys Gly Asp Arg Ala Ser Gly Lys His Tyr Gly Val Tyr Ser  
50 55 60

Cys Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asn Leu  
65 70 75 80

Ser Tyr Ala Cys Arg Glu Glu Asn Lys Cys Ile Ile Asp Lys Arg Gln  
85 90 95

Arg Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Thr Met Gly  
100 105 110

Met Lys Arg Glu Ala Val Gln Glu Glu Arg Gln Arg Thr Lys Glu Arg  
115 120 125

Asp His Asn Asn Ile Glu Val Glu Pro Thr Ser Ser Ser Asn Thr Asp  
130 135 140

Met Pro Val Glu Leu Ile Leu Arg Ala Glu Asn Lys Ala Asp Ala Ile  
145 150 155 160

Lys Thr Glu Gln Gln Tyr Ile Glu Gln Arg His Pro Gln His Thr Val  
165 170 175

Gly Ala Ile Cys Gln Ala Thr Asp Lys Gln Leu Ile Gln Leu Val Glu  
180 185 190

Trp Ala Lys His Ile Pro His Phe Lys Asn Leu Pro Leu Gly Asp Gln  
195 200 205

Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Met Ile Ala Ala Phe  
210 215 220

Ser His Arg Ser Ile Ser Val Lys Asp Gly Ile Val Leu Ala Thr Gly  
225 230 235 240

Leu Thr Val Asp Arg Asp Ser Ala His Gln Ala Gly Val Glu Ala Ile  
245 250 255

Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Asp Met Gly  
260 265 270

Met Asp Arg Thr Glu Leu Gly Cys Leu Arg Thr Ile Ile Leu Phe Asn  
 275 280 285

Pro Gly Ser Lys Gly Leu Gln Ser Val Asn Glu Val Gln Val Leu Arg  
 290 295 300

Asp Lys Val Tyr Val Ala Leu Glu Glu Tyr Cys Arg Thr Thr His Pro  
 305 310 315 320

Glu Glu Pro Gly Arg Phe Ala Lys Leu Leu Arg Leu Pro Ser Leu  
 325 330 335

Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Tyr Lys Leu  
 340 345 350

Ile Gly Asp Ser Pro Ile Asp Thr Phe Leu Met Glu Val Leu Glu Ser  
 355 360 365

Ser Ser His Asp Val Gln Val Ala Thr  
 370 375

<210> 17  
 <211> 1131  
 <212> DNA  
 <213> Myzus persicae

<220>  
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<400> 17  
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 Val Asp Arg Asn Ser Met Met Asn Asn Ser Cys Asn Val Gln Asp Ser  
 20 25 30

ccg aat tac ccg ccc aac cat cca ctc agc ggt tcg aaa cat ctg tgc 144  
 Pro Asn Tyr Pro Pro Asn His Pro Leu Ser Gly Ser Lys His Leu Cys  
 35 40 45

tcc ata tgc ggc gat cgc gcc agt gga aaa cat tac gga gtc tac agc 192  
 Ser Ile Cys Gly Asp Arg Ala Ser Gly Lys His Tyr Gly Val Tyr Ser  
 50 55 60

tgc gag ggg tgc aaa ggg ttc ttc aaa cgc aca gtg agg aaa aat ttg 240  
 Cys Glu Gly Cys Lys Phe Phe Lys Arg Thr Val Arg Lys Asn Leu  
 65 70 75 80

tca	tac	gcg	tgt	cgc	gaa	gaa	aac	aaa	tgc	atc	atc	gac	aag	cgc	caa	288
Ser	Tyr	Ala	Cys	Arg	Glu	Glu	Asn	Lys	Cys	Ile	Ile	Asp	Lys	Arg	Gln	
				85					90					95		
cga	aat	cgg	tgc	caa	tac	tgc	agg	tat	caa	aaa	tgt	ttg	acc	atg	ggc	336
Arg	Asn	Arg	Cys	Gln	Tyr	Cys	Arg	Tyr	Gln	Lys	Cys	Leu	Thr	Met	Gly	
			100					105					110			
atg	aaa	aga	gaa	gct	gtg	cag	gaa	gaa	aga	caa	cgt	aca	aaa	gaa	cga	384
Met	Lys	Arg	Glu	Ala	Val	Gln	Glu	Glu	Arg	Gln	Arg	Thr	Lys	Glu	Arg	
			115				120					125				
gat	cat	aat	aac	atc	gaa	gtt	gaa	ccc	acg	agc	agt	tct	aat	act	gat	432
Asp	His	Asn	Asn	Ile	Glu	Val	Glu	Pro	Thr	Ser	Ser	Ser	Asn	Thr	Asp	
			130				135					140				
atg	cca	gtg	gaa	ctc	ata	tta	agg	gct	gag	aat	aaa	gct	gat	gct	ata	480
Met	Pro	Val	Glu	Leu	Ile	Leu	Arg	Ala	Glu	Asn	Lys	Ala	Asp	Ala	Ile	
					150					155					160	
aag	act	gaa	caa	cag	tat	ata	gag	caa	cga	cat	cct	caa	cat	act	gtt	528
Lys	Thr	Glu	Gln	Gln	Tyr	Ile	Glu	Gln	Arg	His	Pro	Gln	His	Thr	Val	
				165				170						175		
ggg	gct	att	tgt	caa	gca	act	gac	aag	cag	tta	ata	caa	ctt	gtt	gaa	576
Gly	Ala	Ile	Cys	Gln	Ala	Thr	Asp	Lys	Gln	Leu	Ile	Gln	Leu	Val	Glu	
			180					185					190			
tgg	gcc	aag	cat	ata	ccg	cat	ttt	aaa	aat	tta	cct	cta	ggc	gat	caa	624
Trp	Ala	Lys	His	Ile	Pro	His	Phe	Lys	Asn	Leu	Pro	Leu	Gly	Asp	Gln	
			195				200					205				
gtt	tta	tta	ttg	aga	gct	ggg	tgg	aat	gag	ttg	atg	att	gca	gca	ttt	672
Val	Leu	Leu	Leu	Arg	Ala	Gly	Trp	Asn	Glu	Leu	Met	Ile	Ala	Ala	Phe	
			210				215				220					
tcc	cat	aga	tca	atc	agt	gta	aaa	gat	ggg	ata	gtc	tta	gct	act	gga	720
Ser	His	Arg	Ser	Ile	Ser	Val	Lys	Asp	Gly	Ile	Val	Leu	Ala	Thr	Gly	
					230					235					240	
ctt	act	gtt	gac	aga	gat	tca	gct	cac	caa	gct	ggg	gtt	gaa	gct	ata	768
Leu	Thr	Val	Asp	Arg	Asp	Ser	Ala	His	Gln	Ala	Gly	Val	Glu	Ala	Ile	
				245					250					255		
ttt	gat	cgt	gta	ctc	act	gaa	ctc	gtt	gct	aaa	atg	aga	gat	atg	ggg	816
Phe	Asp	Arg	Val	Leu	Thr	Glu	Leu	Val	Ala	Lys	Met	Arg	Asp	Met	Gly	
			260					265					270			
atg	gat	aga	aca	gag	ctt	ggc	tgt	ttg	cgt	act	att	att	ctt	ttt	aat	864
Met	Asp	Arg	Thr	Glu	Leu	Gly	Cys	Leu	Arg	Thr	Ile	Ile	Leu	Phe	Asn	
			275													



cca ggt tca aaa ggt ttg cag tct gtg aat gaa gtg gaa gta ctg cgt 912  
 Pro Gly Ser Lys Gly Leu Gln Ser Val Asn Glu Val Glu Val Leu Arg  
 290 295 300

gat aag gtt tat gtt gcg tta gaa gaa tat tgt cgt aca aca cat cca 960  
 Asp Lys Val Tyr Val Ala Leu Glu Glu Tyr Cys Arg Thr Thr His Pro  
 305 310 315 320

gaa gaa cct gga cga ttt gct aaa cta ctt ctt cgg ctt cct tca tta 1008  
 Glu Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu  
 325 330 335

cgt tca att gga tta aaa tgt ctg gaa cat tta ttc ttt tat aaa ctt 1056  
 Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Tyr Lys Leu  
 340 345 350

att ggc gat tcc cca att gat aca ttt tta atg gaa gtt ctc gaa tca 1104  
 Ile Gly Asp Ser Pro Ile Asp Thr Phe Leu Met Glu Val Leu Glu Ser  
 355 360 365

tct tca cat gac gtt caa gta gct aca 1131  
 Ser Ser His Asp Val Gln Val Ala Thr  
 370 375

<210> 18  
 <211> 377  
 <212> PRT  
 <213> Myzus persicae

<400> 18  
 Met Tyr Ser Asn Ser Tyr Thr Met Tyr Ser Ser Asp Arg Leu Tyr Ser  
 1 5 10 15

Val Asp Arg Asn Ser Met Met Asn Asn Ser Cys Asn Val Gln Asp Ser  
 20 25 30

Pro Asn Tyr Pro Pro Asn His Pro Leu Ser Gly Ser Lys His Leu Cys  
 35 40 45

Ser Ile Cys Gly Asp Arg Ala Ser Gly Lys His Tyr Gly Val Tyr Ser  
 50 55 60

Cys Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asn Leu  
 65 70 75 80

Ser Tyr Ala Cys Arg Glu Glu Asn Lys Cys Ile Ile Asp Lys Arg Gln  
 85 90 95

Arg Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Thr Met Gly  
 100 105 110

100-87963-062002

<210> 19  
 <211> 1242  
 <212> DNA  
 <213> Myzus persicae

<220>  
 <221> CDS  
 <222> (1)..(1239)

<400> 19  
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 1 5 10 15  
 agt tca atg ggt cct cag tcg ccc cta gac ctc aaa cct gac acg gca 96  
 Ser Ser Met Gly Pro Gln Ser Pro Leu Asp Leu Lys Pro Asp Thr Ala  
 20 25 30  
 act tta atg gtt aat ttc agt cct ccg gga gct cct cta agt cct gca 144  
 Thr Leu Met Val Asn Phe Ser Pro Pro Gly Ala Pro Leu Ser Pro Ala  
 35 40 45  
 gga tta tac agc gtc gat cgg aac agt atg atg aat aat tct tgc aac 192  
 Gly Leu Tyr Ser Val Asp Arg Asn Ser Met Met Asn Asn Ser Cys Asn  
 50 55 60  
 gta caa gac tct ccg aat tac ccg ccc aac cat cca ctc agc ggt tcg 240  
 Val Gln Asp Ser Pro Asn Tyr Pro Pro Asn His Pro Leu Ser Gly Ser  
 65 70 75 80  
 aaa cat ctg tgc tcc ata tgc ggc gat cgc gcc agt gga aaa cat tac 288  
 Lys His Leu Cys Ser Ile Cys Gly Asp Arg Ala Ser Gly Lys His Tyr  
 85 90 95  
 gga gtc tac agc tgc gag ggg tgc aaa ggg ttc ttc aaa cgc aca gtg 336  
 Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Val  
 100 105 110  
 agg aaa aat ttg tca tac gcg tgt cgc gaa gaa aac aaa tgc atc atc 384  
 Arg Lys Asn Leu Ser Tyr Ala Cys Arg Glu Glu Asn Lys Cys Ile Ile  
 115 120 125  
 gac aag cgc caa cga aat cgg tgc caa tac tgc agg tat caa aaa tgt 432  
 Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys  
 130 135 140  
 ttg acc atg ggc atg aaa aga gaa gct gtg cag gaa gaa aga caa cgt 480  
 Leu Thr Met Gly Met Lys Arg Glu Ala Val Gln Glu Glu Arg Gln Arg  
 145 150 155 160

aca aaa gaa cga gat cat aat aac atc gaa gtt gaa ccc acg agc agt	528
Thr Lys Glu Arg Asp His Asn Asn Ile Glu Val Glu Pro Thr Ser Ser	
165 170 175	
tct aat act gat atg cca gtg gaa ctc ata tta agg gct gag aat aaa	576
Ser Asn Thr Asp Met Pro Val Glu Leu Ile Leu Arg Ala Glu Asn Lys	
180 185 190	
gct gat gct ata aag act gaa caa cag tat ata gag caa cga cat cct	624
Ala Asp Ala Ile Lys Thr Glu Gln Gln Tyr Ile Glu Gln Arg His Pro	
195 200 205	
caa cat act gtt ggt gct att tgt caa gca act gac aag cag tta ata	672
Gln His Thr Val Gly Ala Ile Cys Gln Ala Thr Asp Lys Gln Leu Ile	
210 215 220	
caa ctt gtt gaa tgg gcc aag cat ata ccg cat ttt aaa aat tta cct	720
Gln Leu Val Glu Trp Ala Lys His Ile Pro His Phe Lys Asn Leu Pro	
225 230 235 240	
cta ggc gat caa gtt tta tta ttg aga gct ggt tgg aat gag ttg atg	768
Leu Gly Asp Gln Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Met	
245 250 255	
att gca gca ttt tcc cat aga tca atc agt gta aaa gat ggt ata gtc	816
Ile Ala Ala Phe Ser His Arg Ser Ile Ser Val Lys Asp Gly Ile Val	
260 265 270	
tta gct act gga ctt act gtt gac aga gat tca gct cac caa gct ggt	864
Leu Ala Thr Gly Leu Thr Val Asp Arg Asp Ser Ala His Gln Ala Gly	
275 280 285	
gtt gaa gct ata ttt gat cgt gta ctc act gaa ctc gtt gct aaa atg	912
Val Glu Ala Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met	
290 295 300	
aga gat atg ggt atg gat aga aca gag ctt ggc tgt ttg cgt act att	960
Arg Asp Met Gly Met Asp Arg Thr Glu Leu Gly Cys Leu Arg Thr Ile	
305 310 315 320	
att ctt ttt aat cca ggt tca aaa ggt ttg cag tct gtg aat gaa gtg	1008
Ile Leu Phe Asn Pro Gly Ser Lys Gly Leu Gln Ser Val Asn Glu Val	
325 330 335	
gaa gta ctg cgt gat aag gtt tat gtt gcg tta gaa gaa tat tgt cgt	1056
Glu Val Leu Arg Asp Lys Val Tyr Val Ala Leu Glu Glu Tyr Cys Arg	
340 345 350	
aca aca cat cca gaa gaa cct gga cga ttt gct aaa cta ctt ctt cgg	1104
Thr Thr His Pro Glu Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg	
355 360 365	

ctt cct tca tta cgt tca att gga tta aaa tgt ctg gaa cat tta ttc 1152  
 Leu Pro Ser Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe  
 370 375 380

ttt tat aaa ctt att ggc gat tcc cca att gat aca ttt tta atg gaa 1200  
 Phe Tyr Lys Leu Ile Gly Asp Ser Pro Ile Asp Thr Phe Leu Met Glu  
 385 390 395 400

gtt ctc gaa tca tct tca cat gac gtt caa gta gct aca tga 1242  
 Val Leu Glu Ser Ser Ser His Asp Val Gln Val Ala Thr  
 405 410

<210> 20  
 <211> 413  
 <212> PRT  
 <213> Myzus persicae

<400> 20  
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 1 5 10 15

Ser Ser Met Gly Pro Gln Ser Pro Leu Asp Leu Lys Pro Asp Thr Ala  
 20 25 30

Thr Leu Met Val Asn Phe Ser Pro Pro Gly Ala Pro Leu Ser Pro Ala  
 35 40 45

Gly Leu Tyr Ser Val Asp Arg Asn Ser Met Met Asn Asn Ser Cys Asn  
 50 55 60

Val Gln Asp Ser Pro Asn Tyr Pro Pro Asn His Pro Leu Ser Gly Ser  
 65 70 75 80

Lys His Leu Cys Ser Ile Cys Gly Asp Arg Ala Ser Gly Lys His Tyr  
 85 90 95

Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Val  
 100 105 110

Arg Lys Asn Leu Ser Tyr Ala Cys Arg Glu Glu Asn Lys Cys Ile Ile  
 115 120 125

Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys  
 130 135 140

Leu Thr Met Gly Met Lys Arg Glu Ala Val Gln Glu Glu Arg Gln Arg  
 145 150 155 160

Thr Lys Glu Arg Asp His Asn Asn Ile Glu Val Glu Pro Thr Ser Ser  
 165 170 175

Ser Asn Thr Asp Met Pro Val Glu Leu Ile Leu Arg Ala Glu Asn Lys  
180 185 190

Ala Asp Ala Ile Lys Thr Glu Gln Gln Tyr Ile Glu Gln Arg His Pro  
195 200 205

Gln His Thr Val Gly Ala Ile Cys Gln Ala Thr Asp Lys Gln Leu Ile  
210 215 220

Gln Leu Val Glu Trp Ala Lys His Ile Pro His Phe Lys Asn Leu Pro  
225 230 235 240

Leu Gly Asp Gln Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Met  
245 250 255

Ile Ala Ala Phe Ser His Arg Ser Ile Ser Val Lys Asp Gly Ile Val  
260 265 270

Leu Ala Thr Gly Leu Thr Val Asp Arg Asp Ser Ala His Gln Ala Gly  
275 280 285

Val Glu Ala Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met  
290 295 300

Arg Asp Met Gly Met Asp Arg Thr Glu Leu Gly Cys Leu Arg Thr Ile  
305 310 315 320

Ile Leu Phe Asn Pro Gly Ser Lys Gly Leu Gln Ser Val Asn Glu Val  
325 330 335

Glu Val Leu Arg Asp Lys Val Tyr Val Ala Leu Glu Glu Tyr Cys Arg  
340 345 350

Thr Thr His Pro Glu Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg  
355 360 365

Leu Pro Ser Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe  
370 375 380

Phe Tyr Lys Leu Ile Gly Asp Ser Pro Ile Asp Thr Phe Leu Met Glu  
385 390 395 400

Val Leu Glu Ser Ser Ser His Asp Val Gln Val Ala Thr  
405 410

<210> 21  
<211> 150  
<212> DNA  
<213> Lucilia cuprina

<220>

<221> CDS

<222> (9)..(134)

<400> 21

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ttg aca tat gct tgt cgt gag gac aga aat tgc att ata gat aaa cga 98  
 Leu Thr Tyr Ala Cys Arg Glu Asp Arg Asn Cys Ile Ile Asp Lys Arg  
 15 20 25 30

caa aga aat cgt tgc cag tat tgt cgc tac caa aag tgatcgatac cgtcga 150  
 Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys  
 35 40

<210> 22

<211> 42

<212> PRT

<213> Lucilia cuprina

<400> 22

Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr  
 1 5 10 15

Tyr Ala Cys Arg Glu Asp Arg Asn Cys Ile Ile Asp Lys Arg Gln Arg  
 20 25 30

Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys  
 35 40

<210> 23

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
 oligonucleotide useful as a primer

<220>

<221> unsure

<222> (1)..(32)

<223> At positions 25 and 28, n is a or g or c or t.

<400> 23

cggaattccg cctcnggnta ycaytayaay gc

32

<210> 24  
 <211> 32  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:  
 oligonucleotide useful as a primer

<400> 24  
 cgcgatccr cactcctgac actttcgyct ca 32

<210> 25  
 <211> 23  
 <212> DNA  
 <213> Artificial Sequence

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 <223> Description of Artificial Sequence:  
 oligonucleotide useful as a primer

<400> 25  
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<210> 26  
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 oligonucleotide useful as a primer

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 oligonucleotide useful as a primer

<400> 27  
 tcgtccggtt accattacaa cgc 23





oligonucleotide useful as a primer

<400> 31  
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<210> 32  
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<210> 35  
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<223> Description of Artificial Sequence:  
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1 5 10 15

aac tgt ctg att gat aag agg caa agg aat cga tgt caa tat 91  
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20 25 30

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 1 5 10 15  
 ggt att ggt ata ata gaa tca tcc gaa tct aac gga gct atc atg ttg 96  
 Gly Ile Gly Ile Ile Glu Ser Ser Glu Ser Asn Gly Ala Ile Met Leu  
 20 25 30  
 aac gat tct aca tct aat tgt cca tca cct tca cca tct cgt gtg gtt 144  
 Asn Asp Ser Thr Ser Asn Cys Pro Ser Pro Ser Pro Ser Arg Val Val  
 35 40 45  
 cat ata cgg aat gtt ccc ata gaa gct act gaa aat gat gtt ctc agt 192  
 His Ile Arg Asn Val Pro Ile Glu Ala Thr Glu Asn Asp Val Leu Ser  
 50 55 60  
 att ggt act cca ttt ggt gag atc acc aat gtt ctt ttg gtg aga gga 240  
 Ile Gly Thr Pro Phe Gly Glu Ile Thr Asn Val Leu Leu Val Arg Gly  
 65 70 75 80  
 aaa ggt caa gcc ttc tta gag ttt gtt gac tca ttc tct gct caa caa 288  
 Lys Gly Gln Ala Phe Leu Glu Phe Val Asp Ser Phe Ser Ala Gln Gln  
 85 90 95  
 atg gtt aac tgt tgg tct gat cct aac aac tca ccg atg caa ctt tgt 336  
 Met Val Asn Cys Trp Ser Asp Pro Asn Asn Ser Pro Met Gln Leu Cys  
 100 105 110  
 att cgg gga aga caa gta tgt gtc cag ttt tca aag cac aaa gaa ctt 384  
 Ile Arg Gly Arg Gln Val Cys Val Gln Phe Ser Lys His Lys Glu Leu  
 115 120 125  
 aaa aaa tct ctt ctt gga aca aat gct ggt tca gac agc agc tat caa 432  
 Lys Lys Ser Leu Leu Gly Thr Asn Ala Gly Ser Asp Ser Ser Tyr Gln  
 130 135 140  
 agt aca tct cct caa aat agt aga cat ata agc aac ggt gat tct gtt 480  
 Ser Thr Ser Pro Gln Asn Ser Arg His Ile Ser Asn Gly Asp Ser Val  
 145 150 155 160

gga gca agt tcc gtt ttc tct aat cca aat cat cct tta agc gga tca	528
Gly Ala Ser Ser Val Phe Ser Asn Pro Asn His Pro Leu Ser Gly Ser	
165 170 175	
aaa cat ctc tgt tct att tgt ggt gat cga gcc tct ggg aaa cat tat	576
Lys His Leu Cys Ser Ile Cys Gly Asp Arg Ala Ser Gly Lys His Tyr	
180 185 190	
ggt gtt tac agt tgt gaa gga tgt aaa gga ttt ttt aaa agg act gtt	624
Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Val	
195 200 205	
cgt aaa gat ttg tct tat gct tgt cgg gaa gaa cga gat tgt atc ata	672
Arg Lys Asp Leu Ser Tyr Ala Cys Arg Glu Glu Arg Asp Cys Ile Ile	
210 215 220	
gac aga cga caa agg aat agg tgt caa tac tgt aga tat cag aaa tgt	720
Asp Arg Arg Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys	
225 230 235 240	
ctc gct atg gga atg aaa aga gaa gcc gtg caa gaa gaa aga caa agg	768
Leu Ala Met Gly Met Lys Arg Glu Ala Val Gln Glu Glu Arg Gln Arg	
245 250 255	
aat aaa gaa aaa agt gaa aac gag gtt gaa agt aca agt aac tca cag	816
Asn Lys Glu Lys Ser Glu Asn Glu Val Glu Ser Thr Ser Asn Ser Gln	
260 265 270	
aat gat atg cct atc gaa aga ata ctg gaa gct gaa tta cga gtg gaa	864
Asn Asp Met Pro Ile Glu Arg Ile Leu Glu Ala Glu Leu Arg Val Glu	
275 280 285	
cct aag aat gaa gac ata gat tct cga gat ccc gtt agt gat atc tgt	912
Pro Lys Asn Glu Asp Ile Asp Ser Arg Asp Pro Val Ser Asp Ile Cys	
290 295 300	
caa gcg gca gat cga caa ctt tac caa tta att gaa tgg gct aag cat	960
Gln Ala Ala Asp Arg Gln Leu Tyr Gln Leu Ile Glu Trp Ala Lys His	
305 310 315 320	
att cct cat ttc acc gag tta ccc gtt gaa gat caa gtt att tta ctt	1008
Ile Pro His Phe Thr Glu Leu Pro Val Glu Asp Gln Val Ile Leu Leu	
325 330 335	
aaa tca gga tgg aat gag ctt ctc att gca ggc ttt tct cat cgt tca	1056
Lys Ser Gly Trp Asn Glu Leu Leu Ile Ala Gly Phe Ser His Arg Ser	
340 345 350	
atg tca gtt aaa gat ggt atc atg tta gcc act ggt ttg gtt gtt cat	1104
Met Ser Val Lys Asp Gly Ile Met Leu Ala Thr Gly Leu Val Val His	
355 360 365	

aga aac tgt gct cat caa gcg ggt gta ggt gct att ttt gat cgc gtg 1152  
 Arg Asn Cys Ala His Gln Ala Gly Val Gly Ala Ile Phe Asp Arg Val  
 370 375 380

tta act gaa tta gtg gct aaa atg aga gaa atg aaa atg gac aaa act 1200  
 Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Lys Thr  
 385 390 395 400

gaa ctt ggt tgc tta cga tct att gta tta ttt aat ccc gaa gct aaa 1248  
 Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro Glu Ala Lys  
 405 410 415

gga ctc aaa tca aca caa caa gtt gaa aat tta cgt gaa aag gtt tac 1296  
 Gly Leu Lys Ser Thr Gln Gln Val Glu Asn Leu Arg Glu Lys Val Tyr  
 420 425 430

gca atc ctg gaa gag tat tgt aga caa act tat cct gat caa tct ggc 1344  
 Ala Ile Leu Glu Glu Tyr Cys Arg Gln Thr Tyr Pro Asp Gln Ser Gly  
 435 440 445

cgt ttt gct aaa tta ctt ctt cgt cta cct gcc tta cgg tca att ggt 1392  
 Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly  
 450 455 460

tta aag tgt ttg gaa cat tta ttt ttc ttc aaa ttg gtt gga aac aca 1440  
 Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Val Gly Asn Thr  
 465 470 475 480

tct att gac agt ttc ttg ttg tcc atg tta gaa tct aat tca gac tca 1488  
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 485 490 495

tag 1491

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 <213> Bemisia tabaci

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 20 25 30

Asn Asp Ser Thr Ser Asn Cys Pro Ser Pro Ser Pro Ser Arg Val Val  
 35 40 45

His Ile Arg Asn Val Pro Ile Glu Ala Thr Glu Asn Asp Val Leu Ser  
 50 55 60

Ile	Gly	Thr	Pro	Phe	Gly	Glu	Ile	Thr	Asn	Val	Leu	Leu	Val	Arg	Gly	65	70	75	80
Lys	Gly	Gln	Ala	Phe	Leu	Glu	Phe	Val	Asp	Ser	Phe	Ser	Ala	Gln	Gln	85	90	95	
Met	Val	Asn	Cys	Trp	Ser	Asp	Pro	Asn	Asn	Ser	Pro	Met	Gln	Leu	Cys	100	105	110	
Ile	Arg	Gly	Arg	Gln	Val	Cys	Val	Gln	Phe	Ser	Lys	His	Lys	Glu	Leu	115	120	125	
Lys	Lys	Ser	Leu	Leu	Gly	Thr	Asn	Ala	Gly	Ser	Asp	Ser	Ser	Tyr	Gln	130	135	140	
Ser	Thr	Ser	Pro	Gln	Asn	Ser	Arg	His	Ile	Ser	Asn	Gly	Asp	Ser	Val	145	150	155	160
Gly	Ala	Ser	Ser	Val	Phe	Ser	Asn	Pro	Asn	His	Pro	Leu	Ser	Gly	Ser	165	170	175	
Lys	His	Leu	Cys	Ser	Ile	Cys	Gly	Asp	Arg	Ala	Ser	Gly	Lys	His	Tyr	180	185	190	
Gly	Val	Tyr	Ser	Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Lys	Arg	Thr	Val	195	200	205	
Arg	Lys	Asp	Leu	Ser	Tyr	Ala	Cys	Arg	Glu	Glu	Arg	Asp	Cys	Ile	Ile	210	215	220	
Asp	Arg	Arg	Gln	Arg	Asn	Arg	Cys	Gln	Tyr	Cys	Arg	Tyr	Gln	Lys	Cys	225	230	235	240
Leu	Ala	Met	Gly	Met	Lys	Arg	Glu	Ala	Val	Gln	Glu	Glu	Arg	Gln	Arg	245	250	255	
Asn	Lys	Glu	Lys	Ser	Glu	Asn	Glu	Val	Glu	Ser	Thr	Ser	Asn	Ser	Gln	260	265	270	
Asn	Asp	Met	Pro	Ile	Glu	Arg	Ile	Leu	Glu	Ala	Glu	Leu	Arg	Val	Glu	275	280	285	
Pro	Lys	Asn	Glu	Asp	Ile	Asp	Ser	Arg	Asp	Pro	Val	Ser	Asp	Ile	Cys	290	295	300	
Gln	Ala	Ala	Asp	Arg	Gln	Leu	Tyr	Gln	Leu	Ile	Glu	Trp	Ala	Lys	His	305	310	315	320
Ile	Pro	His	Phe	Thr	Glu	Leu	Pro	Val	Glu	Asp	Gln	Val	Ile	Leu	Leu	325	330	335	





<210> 42  
 <211> 33  
 <212> PRT  
 <213> Bemisia tabaci

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 1 5 10 15  
 Asn Ala Val Tyr Gln Cys Lys Tyr Gly Asn Asn Cys Glu Ile Asp Met  
 20 25 30

Tyr

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**NOVEL GENETIC SEQUENCES ENCODING STEROID AND JUVENILE HORMONE  
RECEPTOR POLYPEPTIDES AND USES THEREFOR**

**FIELD OF THE INVENTION**

5 The present invention relates generally to novel genetic sequences encoding receptor polypeptides and insecticidal modalities therefor, which insecticidal modalities are based upon non-polypeptide insect hormones and their receptors. More specifically, the present invention provides isolated nucleic acid molecules encoding polypeptides comprising functional steroid hormone and juvenile hormone receptors, in particular isolated nucleic acid molecules which

10 encode polypeptides comprising the *Lucilia cuprina* (sheep blowfly), *Myzus persicae* (aphid) and *Bemisia tabaci* (Silverleaf whitefly) ecdysone receptors and juvenile hormone receptors. In a particularly preferred embodiment, the present invention relates to isolated nucleic acid molecules which encode the *L. cuprina*, *M. persicae*, and *B. tabaci* EcR polypeptide subunits or fragments thereof, or which encode the EcR partner protein (USP polypeptide) subunits of

15 *L. cuprina*, *M. persicae*, and *B. tabaci*. The EcR and USP polypeptides disclosed herein associate to form functional heterodimeric ecdysone receptors or receptor analogues. The present invention further provides the *L. cuprina*, *M. persicae*, and *B. tabaci* EcR proteins or fragments thereof, in addition to providing the *L. cuprina*, *M. persicae*, and *B. tabaci* EcR partner protein (USP polypeptide) subunits of ecdysone receptors, and the *L. cuprina*, *M.*

20 *persicae*, and *B. tabaci* USP polypeptides of the juvenile hormone receptors of these insects. The present invention further relates to the production of functional recombinant insect receptors and recombinant polypeptide subunits thereof and derivatives and analogues thereof. The present invention further relates to the uses of the recombinant receptor and isolated nucleic acid molecules of the present invention in the regulation of gene expression. The

25 present invention further relates to screening systems and methods of identifying insecticidally-active agents which are capable of agonising or antagonising insect receptor function, such as molecules or ligands which associate with steroid receptors or juvenile hormone receptors so as to modify the affinity of said receptors for their cognate *cis*-acting response elements (eg. insect steroid response elements, juvenile hormone response elements) in the genes which

30 they regulate, or alternatively or in addition, which modify the affinity of said receptors for their cellular stimuli (eg. insect steroids or juvenile hormones) or analogues thereof, or alternatively or in addition, which act as insecticides by virtue of their ability to agonise or antagonise the activity of insect hormones, such as by mimicry of a ligand which binds to said receptor or a ligand-binding region thereof. The invention further extends to such compounds or ligands.

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## GENERAL

This specification contains nucleotide and amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are defined by descriptor "SEQ ID NO:" followed by the numeric identifier. For example, SEQ ID NO: 1 refers to the information provided in the numeric indicator field designated <400> 1, etc.

The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

Bibliographic details of the publications referred to in this specification are collected at the end of the description. Reference herein to prior art, including any one or more prior art documents, is not to be taken as an acknowledgment, or suggestion, that said prior art is common general knowledge in Australia or forms a part of the common general knowledge in Australia.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

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Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification,  
5 individually or collectively, and any and all combinations or any two or more of said steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally-equivalent  
10 products, compositions and methods are clearly within the scope of the invention, as described herein.

## BACKGROUND TO THE INVENTION

International Patent Application No WO91/13167 (applicant, The Board of Trustees of Leyland  
15 Stanford University, and hereinafter referred to as WO91/13167) describes the identification, characterization, expression and uses of insect steroid receptors and DNA sequences encoding same and, in particular, the identification, characterization, expression and uses of the steroid receptor of the common fruit fly, *Drosophila melanogaster*.

20 It has been found by the present inventors that the limited homology between the *D. melanogaster* steroid receptor-encoding gene sequences and the steroid receptor -encoding sequences derived from other insects, in particular those derived from diptera such as the Australian sheep blowfly *L. cuprina*; hemiptera such as the aphid *M. persicae*, leaf sucking insects such as the whitefly (*B. tabaci*), scale insects and leaf hoppers; coleoptera; neuroptera;  
25 lepidoptera; and ants, as well as from helminths and protozoa, prevents the routine isolation of DNA sequences encoding steroid receptors or juvenile hormone receptors from these latter-mentioned organisms.

Moreover, the present inventors have discovered that the *D. melanogaster* steroid receptor  
30 described in WO91/13167 is temperature-sensitive, showing reduced activity at temperatures above 30°C, such as at temperatures about 37°C, particularly at low concentrations of the

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receptor. Accordingly, the *D. melanogaster* steroid receptor described in WO91/13167 is of limited utility at physiological temperatures applicable to animal or bacterial cells. Moreover, wherein it is desirable to produce a biologically-active steroid receptor using *in vivo* or *in situ* expression systems, which expression systems routinely utilise cells or tissues in the  
5 temperature range of about 28°C to about 42°C, the *D. melanogaster* steroid receptor is also of limited utility.

In work leading up to the present invention, the present inventors developed a novel screening protocol, involving the utilisation of highly-degenerate oligonucleotide probes and primers  
10 derived from the amino acid sequences of the DNA-binding domains of the *D. melanogaster* and *Chironomus tentans* ecdysone receptor polypeptides, to identify nucleotide sequences encoding novel steroid receptor polypeptides and novel insect juvenile hormone receptor polypeptides. The present inventors have further identified specific regions within these novel polypeptides which are suitable for use in preparing a surprising range of novel steroid receptor  
15 polypeptide derivatives and insect juvenile hormone receptor polypeptide derivatives. The novel steroid receptor polypeptides and novel insect juvenile hormone receptor polypeptides of the present invention, and derivative polypeptides thereof, and assembled steroid receptors and insect juvenile hormone receptors derived from said polypeptides and derivatives, and nucleic acid molecules encoding same as exemplified herein, provide the means for developing a wide  
20 range of insecticidally-active agents, as well as methods for the regulated production of bioactive molecules. In particular, the present invention provides the means for developing specific ligands which bind to and either agonise or antagonise the steroid receptors or juvenile hormone receptors, and/or which bind to polypeptide subunits of said receptors as described herein, thereby functioning as highly-specific insecticides, offering significant commercial and  
25 environmental benefits.

The present inventors have been surprisingly successful in characterizing the ecdysone receptor and juvenile hormone receptor derived from insects of the orders Diptera and Hemiptera, and polypeptide components thereof and functional derivatives of said polypeptides  
30 and receptors, particularly in light of the extreme difficulties in dealing with these organisms. The nature of these molecules was unknown prior to the present invention.

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The various aspects of this invention overcome the problems associated with *Drosophila* ecdysone receptors which lack thermal stability. Moreover, those aspects of the invention pertaining to methods of screening for insecticidally active agents do not involve competition assays which are generally complex, and often inaccurate or difficult to calibrate.

5

#### SUMMARY OF THE INVENTION

One aspect of the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence which encodes or is complementary to a sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide or a bioactive derivative

10 or analogue thereof, wherein said polypeptide:

(i) is selected from the group consisting of the EcR polypeptide of an steroid receptor, the partner protein (USP polypeptide) of an steroid receptor and the USP polypeptide of a juvenile hormone receptor; and

15 (ii) comprises an amino acid sequence that is at least 40% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42.

20 In an alternative embodiment, the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence which encodes or is complementary to a sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide or a bioactive derivative or analogue thereof, wherein said polypeptide:

25 (i) is selected from the group consisting of the EcR polypeptide of an steroid receptor, the partner protein (USP polypeptide) of an steroid receptor and the USP polypeptide of a juvenile hormone receptor; and

(ii) comprises an amino acid sequence that is at least 40% identical to an amino acid sequence encoded by the DNA of insects which is present in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, 30 NM99/04567, NM99/04568, NM00/12580, and NM00/12581.

30 In a further alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a steroid receptor EcR polypeptide and comprises the nucleotide

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sequence set forth in SEQ ID NO: 1, or SEQ ID NO: 13.

In a further alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a steroid receptor USP polypeptide or a juvenile hormone receptor polypeptide and comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41.

In a further alternative embodiment, the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 21, SEQ ID NO: 37, and SEQ ID NO: 41, or a complementary nucleotide sequence to any one of said sequences.

In a further alternative embodiment, the present invention provides an isolated nucleic acid molecule which comprises the nucleotide sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 31, and SEQ ID NO: 32, or a complementary nucleotide sequence thereto.

A second aspect of the present invention provides a method of identifying an isolated nucleic acid molecule which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide comprising the steps of:

(i) hybridising genomic DNA, mRNA or cDNA with a hybridisation-effective amount of one or more probes selected from the group consisting of:

(a) a probe comprising at least 10 contiguous nucleotides in length from a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary nucleotide sequence to any one of said sequences;



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(b) a probe comprising at least 10 contiguous nucleotides in length from cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581; and

5 (c) a hybridisation probe comprising a nucleotide sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary nucleotide sequence  
10 to any one of said sequences, or a homologue, analogue or derivative of any one of said sequences or complementary sequences having at least 40% identity thereto; and

(ii) detecting the hybridisation.

15 In an alternative embodiment, the inventive method comprises the steps of:

(i) annealing to genomic DNA, mRNA or cDNA, one or more PCR primers selected from the group consisting of:

(a) a primer comprising at least 10 contiguous nucleotides in length from a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ  
20 ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary nucleotide  
25 sequence to any one of said sequences;

(b) a primer comprising at least 10 contiguous nucleotides in length from cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581; and

30 (ii) amplifying a nucleotide sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide in a polymerase chain reaction.

In a further alternative embodiment, the inventive method comprises the steps of:

- (i) amplifying a nucleotide sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide in a polymerase chain reaction using one or more PCR primers selected from the group consisting of:
- (a) a primer comprising at least 10 contiguous nucleotides in length from a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary nucleotide sequence to any one of said sequences; and
- (b) a primer comprising at least 10 contiguous nucleotides in length from cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581;
- (ii) hybridising the amplified nucleotide sequence to genomic DNA, mRNA or cDNA with a hybridisation-effective amount of one or more probes selected from the group consisting of:
- (a) a probe comprising at least 10 contiguous nucleotides in length derived from a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary nucleotide sequence to any one of said sequences;
- (b) a probe comprising at least 10 contiguous nucleotides in length derived from a cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568,

In an alternative embodiment, the invention provides a recombinant or isolated polypeptide

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comprising a steroid receptor polypeptide or juvenile hormone receptor polypeptide or a bioactive derivative or analogue thereof, wherein said polypeptide:

- 5 (i) is selected from the group consisting of the EcR polypeptide of a steroid receptor, the partner protein (USP polypeptide) of a steroid receptor and the USP polypeptide of a juvenile hormone receptor; and
- (ii) comprises an amino acid sequence that is at least 40% identical to a polypeptide encoded by cDNA present in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581;

10 wherein said polypeptide is substantially free of naturally-associated insect cell components.

A fifth aspect of the invention provides a cell comprising the subject isolated nucleic acid molecule which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide.

15

In a preferred embodiment, the cell of the present invention expresses the polypeptide encoded by the nucleic acid molecule.

In a preferred embodiment, the cell expresses a steroid receptor polypeptide or a fragment thereof which receptor is capable of binding to an insect steroid or analogue thereof or a candidate insecticidally active agent to form an activated complex, and comprises a nucleic acid sequence encoding a bioactive molecule or a reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex promotes transcription of the nucleic acid sequence, wherein said cell on exposure to insect steroid or an analogue thereof, regulates expression of said bioactive molecule or allows detection of said reporter molecule.

In a further aspect of this invention, there is provided an animal (such as a mammal), microorganism, plant or aquatic organism, containing one or more cells as mentioned above.

30

A further aspect of the present invention provides a method of identifying a modulator of insect

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steroid receptor-mediated gene expression or insect juvenile hormone receptor-mediated gene expression comprising:

- (i) assaying the expression of a reporter gene in the presence of a recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention and a potential modulator; and
- (ii) assaying the expression of a reporter gene in the presence of a recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention and without said potential modulator; and
- (ii) comparing expression of the reporter gene in the presence of the potential modulator to the expression of a reporter gene in the absence of the potential modulator,

wherein said reporter gene is placed operably under the control of a steroid response element (SRE) to which said insect steroid receptor binds or a promoter sequence comprising said SRE.

15 A still further aspect of the invention provides a method of identifying a potential insecticidal compound comprising:

- (i) assaying the binding directly or indirectly of a recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention to a steroid response element (SRE) to which said insect steroid receptor binds, in the presence of a candidate compound; and
- (ii) assaying the binding directly or indirectly of a recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention to a steroid response element (SRE) to which said insect steroid receptor binds, in the absence of said candidate compound; and
- (ii) comparing the binding assayed at (i) and (ii), wherein a difference in the level of binding indicates that the candidate compound possesses potential insecticidal activity.

A still further aspect of the invention provides a method of identifying a candidate insecticidally-active agent comprising the steps of:

- a) expressing an EcR polypeptide of a steroid receptor or a fragment thereof which

A still further aspect of the invention provides a synthetic compound which interacts with the

30 In another aspect of this invention, there is provided a method for the regulated production of a bioactive molecule or a reporter molecule in a cell, said method comprising the steps of

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introducing into said cell:

- a) a nucleotide sequence encoding a steroid receptor or a fragment thereof which is capable of binding an insect steroid or analogue thereof, to form an activated complex; and
- b) a nucleotide sequence encoding said bioactive molecule or reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex regulates transcription of the nucleic acid sequence encoding said bioactive molecule or reporter molecule,

wherein exposing the cell to an insect steroid or analogue thereof regulates expression of the  
10 bioactive molecule or reporter molecule.

## SUMMARY OF SEQUENCE LISTING

- |               |   |
|---------------|---|
| SEQ ID NO: 1: | The nucleotide sequence of the open reading frame of a cDNA molecule which encodes the EcR polypeptide subunit of the <i>L. cuprina</i> ecdysone receptor and amino acid sequence therefor.   |
| SEQ ID NO: 2: | The amino acid sequence of the EcR polypeptide subunit of the <i>L. cuprina</i> ecdysone receptor.  |
| SEQ ID NO: 3: | The nucleotide sequence of the cDNA molecule contained in plasmid pBLU1 which encodes the EcR partner protein (USP polypeptide) subunit of the <i>L. cuprina</i> ecdysone receptor or which encodes the USP polypeptide subunit of the <i>L. cuprina</i> juvenile hormone receptor, and amino acid sequence therefor. |
| SEQ ID NO: 4: | The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the <i>L. cuprina</i> ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the <i>L. cuprina</i> juvenile hormone receptor, encoded by SEQ ID NO: 3.  |
| SEQ ID NO: 5: | The nucleotide sequence of the cDNA molecule from plasmid pLSP5 which encodes the EcR partner protein (USP polypeptide) subunit of the <i>L. cuprina</i> ecdysone receptor or which encodes the USP polypeptide subunit of the <i>L. cuprina</i> juvenile hormone receptor, and amino acid sequence therefor.         |



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- SEQ ID NO: 6: The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the *L. cuprina* ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the *L. cuprina* juvenile hormone receptor, encoded by SEQ ID NO: 5.
- 5 SEQ ID NO: 7: The nucleotide sequence of the cDNA molecule from plasmid pLSP12 which encodes the EcR partner protein (USP polypeptide) subunit of the *L. cuprina* ecdysone receptor or which encodes the USP polypeptide subunit of the *L. cuprina* juvenile hormone receptor, and amino acid sequence therefor.
- 10 SEQ ID NO: 8: The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the *L. cuprina* ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the *L. cuprina* juvenile hormone receptor, encoded by SEQ ID NO: 7.
- SEQ ID NO: 9: The nucleotide sequence of a cDNA molecule which encodes part of the  
15 EcR polypeptide subunit of the *M. persicae* ecdysone receptor and amino acid sequence therefor.
- SEQ ID NO: 10: The amino acid sequence of a part of the EcR polypeptide subunit of the *M. persicae* ecdysone receptor.
- SEQ ID NO: 11: The nucleotide sequence of the EcR probe 1 which is specific for  
20 genetic sequences encoding the EcR polypeptide subunit of aphid ecdysone receptors, in particular the EcR polypeptide subunit of the *M. persicae* ecdysone receptor.
- SEQ ID NO: 12: The nucleotide sequence of the EcR probe 2 sequence which is specific  
25 for genetic sequences encoding the EcR polypeptide subunit of aphid ecdysone receptors, in particular the EcR polypeptide subunit of the *M. persicae* ecdysone receptor.
- SEQ ID NO: 13: The nucleotide sequence of the open reading frame of a cDNA molecule which encodes the EcR polypeptide subunit of the *M. persicae* ecdysone receptor and amino acid sequence therefor.
- 30 SEQ ID NO: 14: The amino acid sequence of the EcR polypeptide subunit of the *M. persicae* ecdysone receptor.

- 5 SEQ ID NO: 15: The nucleotide sequence of the open reading frame of a first cDNA molecule encoding the EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor, and amino acid sequence therefor.
- SEQ ID NO: 16: The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor, encoded by SEQ ID NO: 15.
- 10 SEQ ID NO: 17: The nucleotide sequence of the open reading frame of a second cDNA molecule encoding the EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor, and amino acid sequence therefor.
- 15 SEQ ID NO: 18: The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor, encoded by SEQ ID NO: 17.
- 20 SEQ ID NO: 19: The nucleotide sequence of the open reading frame of a third cDNA molecule encoding the EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor, and amino acid sequence therefor.
- 25 SEQ ID NO: 20: The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor, encoded by SEQ ID NO: 19.
- 30 SEQ ID NO: 21: The nucleotide sequence of a 150 base-pair probe which is specific for genetic sequences encoding the EcR partner protein (USP polypeptide) subunit of *L. cuprina* ecdysone receptor or the USP polypeptide subunit of the *L. cuprina* juvenile hormone receptor, and amino acid sequence

therefor.

SEQ ID NO: 22: The amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 21, comprising amino acid residues 108-149 of the EcR partner protein (USP polypeptide) subunit of the *L. cuprina* ecdysone receptor or amino acid residues 108-149 of the amino acid sequence of the USP polypeptide subunit of the *L. cuprina* juvenile hormone receptor set forth herein as SEQ ID NO: 4.

SEQ ID NO: 23: The nucleotide sequence of the degenerate primer Rdna3.

SEQ ID NO: 24: The nucleotide sequence of the degenerate primer Rdna4.

10 SEQ ID NO: 25: The nucleotide sequence of the primer Mdna1.

SEQ ID NO: 26: The nucleotide sequence of the primer Mdna2.

SEQ ID NO: 27: The nucleotide sequence of the primer AP1.

SEQ ID NO: 28: The nucleotide sequence of the degenerate primer AP2.

SEQ ID NO: 29: The sequence of oligonucleotide SPX5 used to construct plasmid pVPLcEcR.

SEQ ID NO: 30: The sequence of oligonucleotide XPS5 used to construct plasmid pVPLcEcR.

SEQ ID NO: 31: The nucleotide sequence of oligonucleotide A used to construct plasmid pSGDM.

20 SEQ ID NO: 32: The nucleotide sequence of oligonucleotide B used to construct plasmid pSGDM.

SEQ ID NO: 33: The sequence of oligonucleotide A used to construct the expression plasmid pMpEcR.LcUSP.DUAL.

SEQ ID NO: 34: The sequence of oligonucleotide B used to construct the expression plasmid pMpEcR.LcUSP.DUAL.

SEQ ID NO: 35: The sequence of oligonucleotide C used to construct the expression plasmid pMpEcR.USP.DUAL.

SEQ ID NO: 36: The sequence of oligonucleotide D used to construct the expression plasmid pMpEcR.USP.DUAL.

30 SEQ ID NO: 37: The nucleotide sequence of a probe which is specific for genetic sequences encoding the EcR partner protein (USP polypeptide) subunit

of *B. tabaci* ecdysone receptor or the USP polypeptide subunit of the *B. tabaci* juvenile hormone receptor, and amino acid sequence therefor.

SEQ ID NO: 38: The amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 37.

5 SEQ ID NO: 39: The nucleotide sequence of the open reading frame of a cDNA molecule encoding the EcR partner protein (USP polypeptide) subunit of the *B. tabaci* ecdysone receptor or the USP polypeptide subunit of the *B. tabaci* juvenile hormone receptor, and amino acid sequence therefor.

10 SEQ ID NO: 40: The amino acid sequence of the EcR partner protein (USP polypeptide) subunit of the *B. tabaci* ecdysone receptor or the amino acid sequence of the USP polypeptide subunit of the *B. tabaci* juvenile hormone receptor encoded by SEQ ID NO: 39.

15 SEQ ID NO: 41: The nucleotide sequence of a probe which is specific for genetic sequences encoding the EcR polypeptide subunit of *B. tabaci* ecdysone receptor, and amino acid sequence therefor.

SEQ ID NO: 42: The amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 37.

#### BRIEF DESCRIPTION OF THE DRAWINGS

20 **Figure 1** is a graphical representation showing function of the EcR polypeptide subunit of the *L. cuprina* ecdysone receptor *in vivo*. CHO cells were co transfected with:

- (1) one of the following expression plasmids: pSGDmEcR, pSGLcEcR, or the parental expression plasmid pSG5 as a control, at 1 µg/ml;
- (2) plasmid p(EcRE)<sub>7</sub>-CAT (1 µg/ml); and
- 25 (3) an independent reporter plasmid, pPGKLacZ, at 1 µg/ml.

CAT expression was induced with Muristerone A at either 10 µM or 50 µM while control cells received only the carrier ethanol. ELISA kits were used to quantify the synthesis of CAT and β-galactosidase in extracts of cells forty eight hours after transfection. The level of CAT was normalized to the level of β-galactosidase in the same extract. Fold-induction represents the  
 30 normalized values for CAT gene expression in cells transfected with pSGDmEcR, pSGLcEcR or pSG5 in the presence of hormone, relative to the normalized values for CAT gene

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expression in cells transfected with the same plasmid, but in the absence of hormone. The average values of three independent experiments are shown and the error bars indicate standard error of the mean.

5 **Figure 2** is a copy of a graphical representation showing the activity of plasmids pSGLD and pSGDL, containing chimeric EcR polypeptide subunits of insect ecdysone receptors, produced as described in the Examples. Cotransfection assays were performed as described in the Examples using plasmids pSGLD and pSGDL and the CAT reporter plasmid p(EcRE)<sub>7</sub>-CAT (1ug/ml) and an independent reporter, pPGKLacZ at 1 µg/ml each. CAT/β-Gal (%) refers to  
10 CAT reporter activity expressed as a percentage relative to β-galactosidase activity produced by the internal control reporter, pPGKLacZ.

**Figure 3** is a copy of a graphical representation showing the binding activity in extracts of Sf9 and Sf21 cells containing a baculovirus expressing LcEcRDEF and LcUSPDEF, as described  
15 in the Examples. Control cells contained baculovirus expressing β-glucuronidase and CAT only.

**Figure 4** is a graphical representation showing the ecdysteroid binding activities of an *in vitro*-translated *Myzus persicae* EcR (MpEcR) polypeptide, an *in vitro*-translated *Myzus persicae*  
20 USP (MpUSP) polypeptide, and a complex formed by *in vitro*-translated *M. persicae* EcR and USP polypeptides.

**Figure 5** is a copy of a graphical representation showing the expression activity of plasmid pVPLcEcR, encoding a chimeric *L. cuprina* EcR polypeptide, and plasmid pSGLcUSP  
25 encoding the *L. cuprina* EcR partner protein (USP polypeptide), in CV1 cells, in accordance with the description provided in Example 18. The CAT reporter plasmid p(EcRE)<sub>7</sub>-CAT (1ug/ml), and an independent reporter plasmid, pPGKLacZ (1 µg/ml) were used to assay ecdysteroid-dependent gene expression. Data indicate expression of the CAT reporter gene relative to the level of expression of the transfection control β-galactosidase reporter gene. The  
30 symbols + and - indicate the presence or absence, respectively, of the plasmids pVPLcEcR and pSGLcUSP, or the presence (+) or absence (-) of 1 µM Ponasterone A (PonA). Error bars

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indicate the standard error of the mean.

**Figure 6** is a graphical representation showing *in vivo* function of a modified EcR polypeptide subunit of the *M. persicae* ecdysone receptor in CHO cells. The CHO cells were co-transfected with a reporter plasmid p(EcRE)<sub>7</sub>-CAT (1µg/ml) and an expression plasmid selected from the group consisting of pSGDmEcR, pSGMpEcR, pSGDM, pSGMD, and pSG5 (described in the examples), also at 1µg/ml concentration. Data indicate CAT reporter gene expression as determined by ELISA, for cells lacking Muristerone A (open bars) or containing 10µM Muristerone A (filled bars). The level of CAT expression is directly correlated to the concentration of the product of the enzymatic reaction in the assay and was measured as an absorbance at 405nm.

**Figure 7** is a copy of a graphical representation showing the binding of [<sup>3</sup>H] ponasterone A to extracts of Sf9 cells infected with baculovirus expressing the ligand binding regions (i.e. domains D/E/F) of (i) the *M. persicae* EcR polypeptide and the *L. cuprina* EcR partner protein (USP polypeptide); (ii) the *M. persicae* EcR polypeptide and the *M. persicae* EcR partner protein (USP polypeptide); and (iii) the *L. cuprina* EcR polypeptide and the *L. cuprina* EcR partner protein (USP polypeptide). Highly significant binding (i.e. above background) of the ecdysteroid analogue is apparent for all three constructs tested.

20

**Figure 8** is a copy of a graphical representation showing the activity of plasmid pSGDM (Example 19), encoding a chimeric *M. persicae* EcR polypeptide, and plasmid pBKMpUSP1, encoding an *M. persicae* EcR partner protein (USP polypeptide), in CV1 cells. The CAT reporter plasmid p(EcRE)<sub>7</sub>-CAT (1ug/ml) and an internal control reporter plasmid, pPopNLacZ (1 µg/ml) were present in all assays. The symbols + and - indicate the presence or absence, respectively, of plasmids indicated in the figure, or the presence (+) or absence (-) of 10 µM Ponasterone a. Data indicate expression of the CAT reporter gene relative to the level of expression of the independent reporter gene β-galactosidase. Error bars indicate the standard error of the mean.

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## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence which encodes or is complementary to a sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide or a bioactive derivative

5 or analogue thereof, wherein said polypeptide:

(i) is selected from the group consisting of the EcR polypeptide of a steroid receptor, the partner protein (USP polypeptide) of a steroid receptor and the USP polypeptide of a juvenile hormone receptor; and

10 (ii) comprises an amino acid sequence having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42.

15 Accordingly, the isolated nucleic acid molecule of the invention may comprise a fragment of a nucleotide sequence encoding a full-length receptor polypeptide.

It is to be understood that a "fragment" of a nucleotide sequence encoding an EcR polypeptide subunit of a steroid receptor or an EcR partner protein (USP polypeptide) of a steroid receptor  
20 or a USP polypeptide of a juvenile hormone receptor, refers to a nucleotide sequence encoding a part or fragment of such a receptor which is capable of binding or associating with an insect steroid or an analogue thereof, or a candidate insecticidally active compound. Fragments of a nucleotide sequence would generally comprise in excess of twenty contiguous nucleotides derived from the base sequence and may encode one or more domains of a functional insect  
25 steroid receptor or juvenile hormone receptor.

Preferably, the isolated nucleic acid molecule of the invention encodes an ecdysteroid receptor polypeptide. Those skilled in the art are aware that ecdysteroid receptors derived from insects are heterodimeric receptors comprising an EcR polypeptide subunit and an EcR partner protein  
30 (USP polypeptide) (see also Jones and Sharp, 1997). In this regard, the present inventors have discovered that the USP polypeptide of the insect juvenile hormone receptor is structurally-

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identical to the EcR partner protein of the ecdysteroid receptor of the present invention, however juvenile hormone receptors comprise monomers or multimers of the USP polypeptide acting without the EcR polypeptide subunit that is present in the ecdysteroid receptors. Accordingly, the present invention extends equally to nucleotide sequences encoding  
5 polypeptides of both the ecdysteroid receptors and polypeptides of the juvenile hormone receptors of insects.

More preferably, the isolated nucleic acid molecule of the invention encodes an ecdysteroid receptor that is modulated by one or more of the steroids ecdysone, ponasterone A, or  
10 muristerone, or an analogue of an ecdysteroid.

The isolated nucleic acid molecule of the invention may be derived from any organism that contains steroid receptors that are responsive to ecdysteroids or ecdysteroid-like compounds or juvenile hormones, or analogues of such receptor-ligands. Accordingly, the present invention  
15 is not to be limited in any of its embodiments to the particular source of the subject nucleic acid, or polypeptide encoded therefor.

Preferably, the isolated nucleic acid molecule of the invention is derived from insects, helminths (nematodes, cestodes, trematodes), protozoa, and ants, amongst others.  
20

More preferably, the isolated nucleic acid molecule of the invention is derived from an insect selected from the group consisting of diptera, hemiptera, coleoptera, neuroptera, lepidoptera and ants, amongst others. Still more preferably, the isolated nucleic acid molecule of the present invention is derived from aphids, scale insects, leaf hoppers, white fly, and blowflies  
25 such as sheep blowflies.

The present invention does not extend to amino acid sequences comprising the complete EcR polypeptide subunit of the *D. melanogaster* ecdysone receptor as described in WO91/13167. However, this exclusion is made on the understanding that the present invention does  
30 encompass chimeric genes and fusion proteins which include the *D. melanogaster* nucleotide and amino acid sequences, respectively.



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In a particularly preferred embodiment, the isolated nucleic acid molecule of the present invention is derived from the aphid *M. persicae* or alternatively, from the Australian sheep blowfly, *L. cuprina*.

- 5 The ecdysteroid receptor is preferably modulated by one or more of the steroids ecdysone, ponasterone A, or muristerone, or an analogue of an ecdysteroid.

- As used herein, the term "analogue of an ecdysteroid" shall be taken to indicate any compound that binds to one or more polypeptide subunits of an ecdysteroid receptor or the heterodimeric  
10 holoreceptor comprising same or alternatively or in addition, which binds to the USP polypeptide of a juvenile hormone receptor or alternatively or in addition, which binds to a bioactive derivative or analogue of said polypeptides or holoreceptor. The term "analogue of an ecdysteroid" shall further be taken to indicate any compound that modulates the bioactivity of one or more polypeptide subunits of an ecdysteroid receptor or the heterodimeric  
15 holoreceptor comprising same or alternatively or in addition, that modulates the bioactivity of the USP polypeptide of a juvenile hormone receptor or alternatively or in addition, that modulates the bioactivity of a bioactive derivative or analogue of said polypeptides or holoreceptor.

- 20 The present invention is not to be limited in scope to the specific *L. cuprina*, *M. persicae*, and *B. tabaci* nucleotide and amino acid sequences set forth in the accompanying Sequence Listing, and persons skilled in the art will readily be able to identify additional related sequences from other sources using art-recognised procedures, for example using nucleic acid hybridisation and/or polymerase chain reaction essentially as described by Ausubel *et al.* (1992)  
25 and/or McPherson *et al.* (1991) and/or Sambrook *et al.* (1989).

Accordingly, the present invention clearly encompasses isolated nucleic acid molecules which encode or are complementary to isolated nucleic acid molecules which encode the subject EcR polypeptide of a steroid receptor or fragments thereof, or the subject EcR partner protein (USP  
30 polypeptide) of a steroid receptor or the subject USP polypeptide of a juvenile hormone receptor, in addition to derivatives, fragments and analogues thereof which comprise amino

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acid sequences having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42.

5

The present invention clearly extends further to isolated nucleic acid molecules which encode or are complementary to isolated nucleic acid molecules which encode the subject EcR polypeptide of a steroid receptor or fragments thereof, or the subject EcR partner protein (USP polypeptide) of a steroid receptor or the subject USP polypeptide of a juvenile hormone receptor, in addition to derivatives, fragments and analogues thereof which comprise amino acid sequences having at least 40% identity to an amino acid sequence encoded by *L. cuprina*, *M. persicae* or *B. tabaci* cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581.

15

For the purposes of nomenclature, plasmid pLcEcR contains the cDNA encoding the EcR polypeptide subunit of the *Lucilia cuprina* ecdysone receptor. This plasmid was deposited on 1 July, 1999 with the Australian Government Analytical Laboratories at 1 Suakin Street, Pymble, New South Wales 2073, Australia under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and accorded AGAL Accession No. NM99/04566.

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For the purposes of nomenclature, plasmid pLcUSP contains the cDNA encoding the EcR partner protein (USP polypeptide) subunit of the *Lucilia cuprina* ecdysone receptor or the USP polypeptide subunit of the *L. cuprina* juvenile hormone receptor. This plasmid was deposited on 1 July, 1999 with the Australian Government Analytical Laboratories at 1 Suakin Street, Pymble, New South Wales 2073, Australia under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and accorded AGAL Accession No. NM99/04565.

25  
30

For the purposes of nomenclature, plasmid pMpEcR contains the cDNA encoding the EcR

polypeptide subunit of the *Myzus persicae* ecdysone receptor. This plasmid was deposited on 1 July, 1999 with the Australian Government Analytical Laboratories at 1 Suakin Street, Pymble, New South Wales 2073, Australia under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and accorded AGAL Accession No. NM99/04567.

For the purposes of nomenclature, plasmid pMpUSP contains a first cDNA encoding the EcR partner protein (USP polypeptide) subunit of the *Myzus persicae* ecdysone receptor or the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor. This plasmid was deposited on 1 July, 1999 with the Australian Government Analytical Laboratories at 1 Suakin Street, Pymble, New South Wales 2073, Australia under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and accorded AGAL Accession No. NM99/04568.

15 For the purposes of nomenclature, plasmid pMpUSP2 contains a second cDNA encoding the EcR partner protein (USP polypeptide) subunit of the *Myzus persicae* ecdysone receptor or the USP polypeptide subunit of the *M. persicae* juvenile hormone receptor. This plasmid was deposited on 21 June, 2000 with the Australian Government Analytical Laboratories at 1 Suakin Street, Pymble, New South Wales 2073, Australia under the provisions of the Budapest Treaty  
20 on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and accorded AGAL Accession No. NM00/12581.

For the purposes of nomenclature, plasmid pBtUSP contains the cDNA encoding the EcR partner protein (USP polypeptide) subunit of the *Bemisia tabaci* ecdysone receptor or the USP polypeptide subunit of the *B. tabaci* juvenile hormone receptor. This plasmid was deposited on 21 June, 2000 with the Australian Government Analytical Laboratories at 1 Suakin Street, Pymble, New South Wales 2073, Australia under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and accorded AGAL Accession No. NM00/12580.

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The deposits referred to herein will be maintained under the Budapest Treaty on the

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International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits are provided merely for the purposes of exemplification and are not an admission that a deposit is required under 35USC §112. A license may be required to make, use or sell the deposited materials or a polypeptide encoded by a cDNA thereof and no such  
5 license is hereby granted. It is to be understood however, that the deposits will become publicly available upon the grant of a patent pertaining to the instant disclosure in so far as that patent relates to the deposits referred to herein.

Preferably, the percentage similarity to any one of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO:  
10 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 40, or SEQ ID NO: 42, or to a polypeptide encoded by a cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581, is at least about 60%, more preferably at least about 80%, even more  
15 preferably at least about 90%.

In determining whether or not two amino acid sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences will arise  
20 in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage identity or similarity between two or more amino acid sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. For example, amino acid sequence identities or similarities  
25 may be calculated using the GAP programme and/or aligned using the PILEUP programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984). The GAP programme utilizes the algorithm of Needleman and Wunsch (1970) to maximise the number of identical/similar residues and to minimise the number and length of sequence gaps in the alignment. Alternatively or in addition,  
30 wherein more than two amino acid sequences are being compared, the ClustalW programme of Thompson *et al* (1994) is used.

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In an alternative embodiment, the isolated nucleic acid molecule of the invention encodes or is complementary to an isolated nucleic acid molecule which encodes a steroid receptor polypeptide or a fragment thereof, or a partner protein (USP) or a fragment thereof, which at least comprises an amino acid sequence which is substantially identical to a sequence  
 5 selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42, or substantially identical to the amino acid sequence of a polypeptide encoded by cDNA contained in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567,  
 10 NM99/04568, NM00/12580, and NM00/12581.

As used herein, the term "substantially identical" or similar term shall be taken to include any sequence which is at least about 95% identical and preferably at least 99% or 100% identical to a stated nucleotide sequence or amino acid sequence, including any homologue, analogue  
 15 or derivative of said stated nucleotide sequence or amino acid sequence.

Those skilled in the art will be aware that variants of the nucleotide sequences set forth in any one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37,  
 20 SEQ ID NO: 39, or SEQ ID NO: 41, or variants of the cDNAs contained in any one of the deposited plasmids, which variants encode EcR polypeptides of insect steroid receptors or fragments thereof or EcR partner proteins (USP polypeptides) or fragments thereof, or USP polypeptides of insect juvenile hormone receptors, may be isolated by hybridization under low stringency conditions as exemplified herein.

25

Such variants include any genomic sequences, cDNA sequences mRNA or other isolated nucleic acid molecules derived from the nucleic acid molecules exemplified herein by the Sequence Listing. Additional variants are not excluded.

30 In a particularly preferred embodiment of the invention, the variant nucleotide sequences encode a fragment of the EcR polypeptide of the insect steroid receptor or a fragment of the

Homologues, analogues and derivatives of the nucleotide sequences exemplified herein may be isolated by hybridising same under at least low stringency conditions and preferably under at least medium stringency conditions, to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary strand of any one of said sequences, or to a cDNA contained in any one or more of the deposited plasmids. More preferably, the isolated nucleic acid molecule according to this aspect of the invention is capable of hybridising under at least high stringency conditions to a nucleic acid molecule selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or to a complementary strand of any one of said sequences, or to the cDNAs contained in any one or more of the deposited plasmids.

- 30 -

For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C or alternatively, as exemplified herein. Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. A medium stringency comprises a hybridisation and/or a wash carried out in 0.2xSSC-2xSSC buffer, 0.1% (w/v) SDS at 42°C to 65°C, while a high stringency comprises a hybridisation and/or a wash carried out in 0.1xSSC-0.2xSSC buffer, 0.1% (w/v) SDS at a temperature of at least 55°C. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of further clarification only, reference to the parameters affecting hybridisation between nucleic acid molecules is found in Ausubel *et al.* (1992), which is herein incorporated by reference.

In an even more preferred embodiment of the invention, a hybridising nucleic acid molecule further comprises a sequence of nucleotides which is at least 40% identical to at least 10 contiguous nucleotides, preferably at least 50 contiguous nucleotides and more preferably at least 100 contiguous nucleotides, derived from a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary strand of any one of said sequences, or a nucleotide sequence of a cDNA contained in any one or more of the deposited plasmids referred to herein.

In determining whether or not two nucleotide sequences fall within these percentage limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage identity between two or more nucleotide sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art. For example, nucleotide sequences may be aligned and their identity calculated using the BESTFIT programme or other appropriate programme of the Computer Genetics Group, Inc., University

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Research Park, Madison, Wisconsin, United States of America (Devereaux *et al*, 1984).

In an alternative embodiment, nucleotide sequences encoding EcR polypeptide subunits of insect steroid receptors or fragments thereof or EcR partner proteins (USP polypeptides) of insect steroid receptor or fragments thereof, or USP polypeptides of insect juvenile hormone receptor polypeptides, are amplified in the polymerase chain reaction. According to this embodiment, one or two or more nucleic acid "primer molecules" derived from a nucleotide sequence exemplified herein, such as, for example, a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary sequence to any one of said sequences, or a sequence from a cDNA contained in any one or more of the deposited plasmids referred to herein, are annealed or hybridized to a nucleic acid "template molecule" which at least comprises a nucleotide sequence encoding a related genetic sequence or a functional part thereof, and nucleic acid molecule copies of the template molecule are amplified enzymatically using a thermostable DNA polymerase enzyme, such as *TaqI* polymerase or *Pfu* polymerase, amongst others.

More particularly, one of the primer molecules comprises contiguous nucleotides derived from a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or from a cDNA contained in any one or more of the deposited plasmids referred to herein; and another of said primers comprises contiguous nucleotides complementary to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or alternatively, from a cDNA contained in any one or more of the deposited plasmids referred to herein, subject to the proviso that the first and second primers are not complementary to each other.

30

In a preferred embodiment, each nucleic acid primer molecule is at least 10 nucleotides in



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length, more preferably at least 20 nucleotides in length, even more preferably at least 30 nucleotides in length, still more preferably at least 40 nucleotides in length and even still more preferably at least 50 nucleotides in length.

5 Furthermore, the nucleic acid primer molecules consists of a combination of any of the nucleotides adenine, cytidine, guanine, thymidine, or inosine, or functional analogues or derivatives thereof which are at least capable of being incorporated into a polynucleotide molecule without having an inhibitory effect on the hybridisation of said primer to the template molecule in the environment in which it is used.

10

Furthermore, one or both of the nucleic acid primer molecules may be contained in an aqueous mixture of other nucleic acid primer molecules, for example a mixture of degenerate primer sequences which vary from each other by one or more nucleotide substitutions or deletions. Alternatively, one or both of the nucleic acid primer molecules may be in a substantially pure

15 form.

In a particularly preferred embodiment exemplified herein, two primer nucleotide sequences are used to amplify related sequences, said primers comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 23 to 30 inclusive. Even more preferably, the primers

20 are used in a primer combination selected from the group consisting of (i) SEQ ID NO: 23 and SEQ ID NO: 24; (ii) SEQ ID NO: 25 and SEQ ID NO: 26; (iii) SEQ ID NO: 27 and SEQ ID NO: 28; and (iv) SEQ ID NO: 31 and SEQ ID NO: 32.

The nucleic acid template molecule may be in a recombinant form, in a virus particle, insect cell, 25 bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the nucleic acid template molecule is derived from an insect species.

Those skilled in the art will be aware that there are many known variations of the basic polymerase chain reaction procedure. Such variations are discussed, for example, in 30 McPherson *et al* (1991). The present invention extends to the use of all such variations in the isolation of variant insect steroid receptor-encoding genes or fragments thereof, or variant

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partner protein-encoding genes or fragments thereof to those exemplified herein.

The isolated nucleic acid molecule of the present invention, including those sequences exemplified herein and any variants thereof, may be cloned into a plasmid or bacteriophage molecule, for example to facilitate the preparation of primer molecules or hybridisation probes or for the production of recombinant gene products. Methods for the production of such recombinant plasmids, cosmids, bacteriophage molecules or other recombinant molecules are well-known to those of ordinary skill in the art and can be accomplished without undue experimentation. Accordingly, the invention further extends to any recombinant plasmid, bacteriophage, cosmid or other recombinant molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41, or a complementary sequence to any one of said sequences, or a homologue, analogue or derivative of any one of said sequences or complements, or a cDNA contained in any one or more of the deposited plasmids referred to herein.

The nucleic acid molecule of the present invention is also useful for developing genetic constructs which comprise and preferably, express, the EcR polypeptide subunit of the insect steroid receptor and/or the EcR partner protein (USP polypeptide) of the steroid receptor or the USP polypeptide of the juvenile hormone receptor, thereby providing for the production of the recombinant polypeptides in isolated cells or transformed tissues.

25

Accordingly, a further aspect of the present invention provides a genetic construct comprising the subject isolated nucleic acid molecule encoding the insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide, operably linked to a promoter sequence. Preferably, the subject nucleic acid molecule is in an expressible format, such that it is possible to produce a recombinant polypeptide therefrom.

30

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Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation in a eukaryotic cell, with or without a CCAAT box sequence or alternatively, the Pribnow box required for accurate expression in prokaryotic cells.

Promoters may be cell, tissue, organ or system specific, or may be non-specific. Using specific promoters, the expression of a bioactive agent or other polypeptide encoded by a structural gene to which the promoter is operably connected may be targeted to a desired cellular site.

For example, in transgenic animals such as sheep, it can be envisaged that cells of the transgenic animal may contain a gene encoding a steroid receptor, preferably a steroid receptor linked to an epidermal specific promoter and a separate gene encoding, for example, epidermal growth factor (EGF) which is functionally linked to one or more insect hormone response elements and may or may not also be linked to epidermal specific promoter elements. On administration of the appropriate insect steroid hormone to the transgenic animal, the activated complex between the insect steroid receptor and insect steroid may bind to the one or more insect steroid hormone response element thereby inducing EGF production solely in epidermal cells which may give rise to defleecing. It is to be understood that this aspect of the invention is independent of the degree of thermostability of the insect steroid receptor. The same principal applies to expression of any bioactive molecule or reporter molecule in a specific cell type which is regulated by a transactivating complex between a steroid receptor complex and an appropriate insect steroid.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression in a cell in response to an external stimulus. Accordingly, the promoter may include further regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Preferred promoters may contain copies of one or more specific regulatory elements, in particular steroid responsive elements (SREs) or hormone-responsive elements (HREs), to further enhance expression and/or to alter the spatial expression and/or temporal expression pattern.

30 Placing a gene or isolated nucleic acid molecule operably under the control of a promoter  
sequence means positioning said gene or isolated nucleic acid molecule such that its

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expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Those skilled in the art will recognise that the choice of promoter will depend upon the nature of the cell being transformed and when expression is required. Furthermore, it is well-known in the art that the promoter sequence used in the expression vector will also vary depending upon the level of expression required and whether expression is intended to be constitutive or regulated.

For expression in eukaryotic cells, the genetic construct generally comprises, in addition to the nucleic acid molecule of the invention, a promoter and optionally other regulatory sequences designed to facilitate expression of said nucleic acid molecule. The promoter may be derived from a genomic clone which normally encodes the expressed protein or alternatively, it may be a heterologous promoter derived from another genetic source. Promoter sequences suitable for expression of genes in eukaryotic cells are well-known in the art.

Suitable promoters for use in eukaryotic expression vectors include those capable of regulating expression in mammalian cells, insect cells such as Sf9 or Sf21. (*Spodoptera frugiperda*) cells, yeast cells and plant cells. Preferred promoters for expression in eukaryotic cells include the p10 promoter, MMTV promoter, polyhedron promoter, the SV40 early promoter and the cytomegalovirus (CMV- IE) promoter, promoters derived from immunoglobulin-producing cells (see, United States Patent No 4,663,281), polyoma virus promoters, and the LTR from various retroviruses (such as murine leukemia virus, murine or Rous sarcoma virus and HIV), amongst



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The prerequisite for expression in prokaryotic cells such as *Escherichia coli* is the use of a strong promoter with an effective ribosome binding site. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, the *lacz* promoter, temperature-sensitive  $\lambda_L$  or  $\lambda_R$  promoters, T7 promoter or the IPTG-inducible *tac* promoter. A number of other vector systems for expressing the nucleic acid molecule of the invention in *E. coli* are well-known in the art and are described for example in Ausubel *et al* (1992).

Numerous vectors having suitable promoter sequences for expression in bacteria have been described, such as for example, pKC30 ( $\lambda_L$ ; Shimatake and Rosenberg, 1981), pKK173-3 (*tac*: Amann and Brosius, 1985), pET-3 (T7: Studier and Moffat, 1986) or the pQE series of expression vectors (Qiagen, CA), amongst others.

Suitable prokaryotic cells include corynebacterium, salmonella, *Escherichia coli*, *Bacillus* sp. and *Pseudomonas* sp, amongst others. Bacterial strains which are suitable for the present purpose are well-known in the relevant art (Ausubel *et al*, 1992).

The genetic constructs described herein may further comprise genetic sequences corresponding to a bacterial origin of replication and/or a selectable marker gene such as an antibiotic-resistance gene, suitable for the maintenance and replication of said genetic construct in a prokaryotic or eukaryotic cell, tissue or organism. Such sequences are well-known in the art.

Selectable marker genes include genes which when expressed are capable of conferring resistance on a cell to a compound which would, absent expression of said selectable marker gene, prevent or slow cell proliferation or result in cell death. Preferred selectable marker genes contemplated herein include, but are not limited to antibiotic-resistance genes such as those conferring resistance to ampicillin, Claforan, gentamycin, G-418, hygromycin, rifampicin, kanamycin, neomycin, spectinomycin, tetracycline or a derivative or related compound thereof or any other compound which may be toxic to a cell.

30

The origin of replication or a selectable marker gene will be spatially-separated from those

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genetic sequences which encode the recombinant receptor polypeptide or fusion polypeptide comprising same.

Preferably, the genetic constructs of the invention, including any expression vectors, are  
5 capable of introduction into, and expression in, an *in vitro* cell culture, or for introduction into, with or without integration into the genome of a cultured cell, cell line or transgenic animal.

In a particularly preferred embodiment, the expression vector is selected from the group consisting of: pLcEcR (AGAL Accession No. NM99/04566); pLcUSP (AGAL Accession No. NM99/04565); pMpEcR (AGAL Accession No. NM99/04567); pMpUSP (AGAL Accession No.  
10 NM99/04568.); pMpUSP2 (AGAL Accession No. NM00/12581); and pBtUSP (AGAL Accession No. NM00/12580).

A further aspect of the invention provides a cell comprising the subject isolated nucleic acid molecule which encodes a steroid receptor polypeptide or a juvenile hormone receptor  
15 polypeptide.

As used herein, the word "cell" shall be taken to refer to a single cell, or a cell lysate, or a tissue, organ or whole organism comprising same, including a tissue, organ or whole organism comprising a clonal group of cells or a heterogenous mixture of cell types, which may be a  
20 prokaryotic or eukaryotic cell as described *supra*.

In a preferred embodiment, the cell of the present invention expresses the isolated or recombinant polypeptide encoded by the nucleic acid molecule.

25 In a preferred embodiment, the cell expresses a steroid receptor polypeptide or a fragment thereof which receptor is capable of binding to an insect steroid or analogue thereof or a candidate insecticidally active agent to form an activated complex, and comprises a nucleic acid sequence encoding a bioactive molecule or a reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex promotes  
30 transcription of the nucleic acid sequence, wherein said cell on exposure to insect steroid or an analogue thereof, regulates expression of said bioactive molecule or allows detection of said



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reporter molecule.

To produce the cells of the invention, host cells are transfected or co-transfected or transformed with nucleotide sequences containing the DNA segments of interest (for example, the insect  
5 steroid receptor gene, the recombinant steroid response elements, or both) by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas lipofection or calcium phosphate treatment are often used for other cellular hosts. See, generally, Sambrook *et al*, (1989); Ausubel *et al*, (1992); and Potrykus (1990). Other transformation techniques include  
10 electroporation, DEAE-dextran, microprojectile bombardment, lipofection, microinjection, and others.

As used herein, the term "transformed cell" is meant to also include the progeny of a transformed cell.

15

In a further aspect of this invention, there is provided an animal (such as a mammal or insect), microorganism, plant or aquatic organism, containing one or more cells as mentioned above. Reference to plants, microorganisms and aquatic organisms includes any such organisms.

20 In this embodiment of the invention, it is to be appreciated that administration of an insect steroid or an analogue thereof to an organism will induce expression of the desired bioactive molecule, such as a polypeptide, with attendant advantages. For example, an induced protein may have a therapeutic effect ameliorating a disease state or preventing susceptibility to disease or may modify in some way the phenotype of an organism to produce a desired effect.  
25 In humans, for example, cell transplants (such as liver cells) may under the action of insect steroids, produce desirable hormones such as insulin, growth hormone, growth factors and the like.

A further aspect of the invention provides a recombinant or isolated polypeptide comprising a  
30 steroid receptor polypeptide or juvenile hormone receptor polypeptide derived from an insect or a bioactive derivative or analogue thereof, wherein said polypeptide:

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- (i) is selected from the group consisting of the EcR polypeptide of a steroid receptor, the partner protein (USP polypeptide) of a steroid receptor and the USP polypeptide of a juvenile hormone receptor; and
- (ii) comprises an amino acid sequence having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42; wherein said polypeptide is substantially free of naturally-associated insect cell components.
- 10 In an alternative embodiment, the recombinant or isolated polypeptide comprising a steroid receptor polypeptide or juvenile hormone receptor polypeptide derived from an insect or a bioactive derivative or analogue thereof, wherein said polypeptide:
- (i) is selected from the group consisting of the EcR polypeptide of a steroid receptor, the partner protein (USP polypeptide) of a steroid receptor and the USP polypeptide of a juvenile hormone receptor; and
- (ii) comprises an amino acid sequence having at least 40% identity to an amino acid sequence encoded by cDNA present in a plasmid selected from the group consisting of AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, NM99/04568, NM00/12580, and NM00/12581;
- 20 wherein said polypeptide is substantially free of naturally-associated insect cell components.

Reference herein to "substantially free of naturally associated insect cell components" refers to at least 80% purity, preferably more than 90% purity, and more preferably more than 95% purity. Normally, purity is measured on a polyacrylamide gel with homogeneity determined by staining of protein bands. Alternatively, high resolution may be necessary using HPLC or similar means. For most purposes, a simple chromatography column or polyacrylamide gel may be used to determine purity. A protein which is chemically synthesized or synthesized in a cell system different from an insect cell from which it naturally originates would be free of naturally-associated insect cell components.

30

The present invention clearly provides for the isolation of EcR polypeptide subunits and EcR

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partner protein (USP polypeptide) subunits of ecdysteroid receptors and USP polypeptides of juvenile hormone receptors, from various organisms of the class *Insecta*, as described *supra*, in addition to protozoa and helminth sources.

5 Insect steroid receptors are characterized by functional ligand-binding domains, and DNA-binding domains, both of which interact to effect a change in the regulatory state of a gene operably linked to the DNA-binding site of the holoreceptor or a polypeptide or polypeptide fragment thereof. Thus, insect steroid receptors seem to be ligand-responsive transcription factors. Additionally, insect steroid receptors generally contain a DNA-binding domain (Domain  
10 C), and a ligand-binding domain (Domain E), separated and flanked by additional domains as identified by Krust *et al* (1986). The C domain preferably comprises a zinc-finger DNA-binding domain which is usually hydrophilic, having high cysteine, lysine and arginine content. The E domain preferably comprises hydrophobic amino acid residues and is further characterized by regions E1, E2 and E3. The ligand-binding domain of the members of the insect steroid  
15 receptor superfamily is typically carboxyl-proximal, relative to a DNA-binding domain (Evans, 1988). The entire ligand-binding domain is typically between about 200 and 250 amino acids but is potentially shorter. This domain has the subregions of high homology, designated the E1, E2 and E3 regions - which may be collectively referred to as the "E region". Amino acid residues proximal to the C domain comprise a region initially defined as separate A and B  
20 domains. Region D separates the more conserved domains C and E. Region D typically has a hydrophilic region whose predicted secondary structure is rich in turns and coils. The F region is carboxy proximal to the E region (see, Krust *et al*, *supra*).

The receptor polypeptides of the present invention exhibit at least a ligand-binding domain, as  
25 characterized by sequence homology to regions E1, E2 and E3. The ligand-binding domains of the present invention are typically characterized by having significant homology in sequence and structure to these three regions. Fragments of insect steroid receptors and partner proteins capable of binding insect steroids, and candidate insecticidally active compounds comprise an E-region or a sufficient portion of the E-region to allow binding.

30

Preferably, the recombinant or isolated EcR polypeptide subunit of the insect steroid receptor

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or EcR partner protein (USP polypeptide) subunit of the steroid receptor or USP polypeptide of the juvenile hormone receptor as described herein is thermostable.

By "thermostable" is meant that a stated integer does not exhibit reduced activity at bacterial,  
5 plant or animal physiological temperatures above about 28°C or above about 30°C. The thermostability of insect steroid hormone receptors also refers to the capacity of such receptors to bind to ligand-binding domains or regions or to transactivate genes linked to insect steroid hormone response elements at bacterial, plant or animal physiological temperatures above about 28°C or above about 30°C.

10

The present invention clearly extends to variants of said polypeptides, as described *supra*. The polypeptide may be substantially free of naturally associated insect cell components, or may be in combination with a partner protein which associates with the insect steroid receptor so as to confer enhanced affinity for insect steroid response elements, enhanced affinity for insect  
15 steroids or analogues thereof. For Example, the amino acid sequences exemplified herein may be varied by the deletion, substitution or insertion of one or more amino acids.

In one embodiment, amino acids of a polypeptide exemplified herein may be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic  
20 moment, charge or antigenicity, and so on.

Substitutions encompass amino acid alterations in which an amino acid of the base polypeptide is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue  
25 contained in the base polypeptide is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which  
30 an amino acid residue which is present in the base polypeptide is substituted with an amino acid having different properties, such as a naturally-occurring amino acid from a different group

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(eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Those skilled in the art will be aware that several means are available for producing variants  
 5 of the exemplified EcR polypeptide subunit of the insect steroid receptor or EcR partner protein (USP polypeptide) subunit of the steroid receptor or USP polypeptide of the juvenile hormone receptor, when provided with the nucleotide sequence of the nucleic acid molecule which encodes said polypeptide, for example site-directed mutagenesis of DNA and polymerase chain reaction utilising mutagenised oligonucleotide primers, amongst others.

10

Such polypeptide variants which are capable of binding insect steroids clearly form part of the present invention. Assays to determine such binding may be carried out according to procedures well known in the art.

15 One such variant polypeptide encompassed by the present invention comprises an "in-frame" fusion polypeptide between different regions of different insect receptor polypeptides. As exemplified herein, the present inventors have discovered that, by producing synthetic genes in which various domains of a base insect steroid receptor-encoding nucleotide sequence derived from a first source are interchanged or substituted with similar sequences derived from  
 20 a second source (referred to as "domain swapping"), it is possible to modify the bioactivity of the insect steroid receptor encoded therefor. For example, the biological activity of the EcR polypeptide of the *L. cuprina* or *M. persicae* ecdysone receptor exemplified herein may be modulated by replacing portions of its C-terminal or N-terminal sequences with the equivalent domains from the EcR polypeptide of the *D. melanogaster* ecdysone receptor or alternatively,  
 25 by swapping regions of the EcR polypeptides of the *L. cuprina* and *M. persicae* ecdysone receptors *per se*.

As a further refinement, such changes in biological function can similarly be effected by making specific changes (e.g. addition, substitution or deletion) to only those amino-acids within each  
 30 domain that are critical for determining the relevant catalytic function (eg. ligand-binding activity, DNA binding site affinity, etc), such as by site-directed mutagenesis.

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According to this embodiment, there is provided a synthetic EcR polypeptide subunit of a steroid receptor, or a synthetic EcR partner protein (USP polypeptide) subunit of a steroid receptor, or a synthetic USP polypeptide of a juvenile hormone receptor, or an analogue or derive of said synthetic polypeptides, wherein said synthetic polypeptides comprise an amino

5 acid sequence which has the following properties:

- (i) it differs in amino acid sequence or exhibits different biological properties to a naturally-occurring EcR polypeptide subunit of a steroid receptor, or a naturally-occurring EcR partner protein (USP polypeptide) subunit of a steroid receptor, or a naturally-occurring USP polypeptide of a juvenile hormone receptor;
- 10 (ii) it comprises a first sequence of amino acids having at least about 40% identity to a part of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42, or having at least about 40% identity to a part of an amino acid sequence
- 15 encoded by any one of the deposited plasmids referred to herein, linked covalently to a second sequence of amino acids derived from an EcR polypeptide subunit of a steroid receptor, EcR partner protein (USP polypeptide) subunit of a steroid receptor, or USP polypeptide of a juvenile hormone receptor, wherein said first and second sequences are derived from different genomic sources.

20

Preferably, the first sequence of amino acids is derived from the EcR polypeptide subunit of a steroid receptor, more preferably from the EcR polypeptide of the *L. cuprina* or *M. persicae* ecdysone receptor, and even more preferably from the EcR polypeptide of the *L. cuprina* ecdysone receptor.

25

In one embodiment, the synthetic EcR polypeptide subunit of a steroid receptor, or a synthetic EcR partner protein (USP polypeptide) subunit of a steroid receptor, or a synthetic USP polypeptide of a juvenile hormone receptor comprises a fusion polypeptide in which the ligand-binding regions of an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42 are

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In order to produce a fusion polypeptide, the nucleic acid molecule which encodes the polypeptide of the invention, or an analogue or derivative thereof, is cloned adjacent to a second nucleic acid molecule encoding the second polypeptide, optionally separated by a spacer nucleic acid molecule which encodes one or more amino acids (eg: poly-lysine or poly  
5 histidine, amongst others), such that the first coding region and the second coding region are in the same open reading frame, with no intervening stop codons between the two coding regions. When translated, the polypeptide thus produced comprises a fusion between the polypeptide products of the first and second coding regions. Wherein a spacer nucleic acid molecule is utilised in the genetic construct, it may be desirable for said spacer to at least  
10 encode an amino acid sequence which is cleavable to assist in separation of the fused polypeptide products of the first and second coding regions, for example a thrombin cleavage site.

A genetic construct which encodes a fusion polypeptide further comprises at least one start  
15 codon and one stop codon, capable of being recognised by the cell's translational machinery in which expression is intended.

Preferably, a genetic construct which encodes a fusion polypeptide may be further modified to include a genetic sequence which encodes a targeting signal placed in-frame with the coding  
20 region of the nucleotide sequence encoding the fusion polypeptide, to target the expressed recombinant polypeptide to the extracellular matrix or other cell compartment. More preferably, the genetic sequence encoding targeting signal is placed in-frame at the 5'-terminus or the 3'-terminus, but most preferably at the 5'-terminus, of the coding region of the nucleotide sequence which encodes the fusion polypeptide.

25

Methods for the production of a fusion polypeptide are well-known to those skilled in the art.

The recombinant EcR polypeptide subunit of the insect steroid receptor or EcR partner protein (USP polypeptide) subunit of the steroid receptor or USP polypeptide of the juvenile hormone  
30 receptor may be purified by standard techniques, such as column chromatography (using various matrices which interact with the protein products, such as ion exchange matrices,





Accordingly, a still further aspect of the invention provides a method of identifying a candidate insecticidally-active agent comprising the steps of:

- a) expressing a USP polypeptide of a juvenile hormone receptor or a fragment thereof which includes the ligand-binding region, optionally in association with an EcR polypeptide of a steroid receptor or ligand binding domain thereof, and optionally in association with an insect steroid or analogue thereof, so as to form a complex;
- b) purifying or precipitating the complex;
- c) determining the three-dimensional structure of the ligand binding domain of the complex; and
- d) identifying compounds which bind to or associate with the three-dimensional structure of the ligand binding domain, wherein said compounds represent candidate insecticidally-active agents.

- Standard procedures are used to determine the three dimensional structure of the receptor polypeptides of the invention, for example using X-ray crystallography and/or nuclear magnetic resonance analysis (see, for example, Bugg *et al.*, 1993; Von Itstein *et al.*, 1993).

Insecticidally-active agents contemplated herein include synthetic chemicals that mimic one or more ligands of the holoreceptor or its polypeptide subunit, or the ligand-binding region of said holoreceptor or subunit, thereby modulating binding of steroids to said holoreceptor or subunit. Preferred insecticidally-active agents include bisacylhydrazines, iridoid glycosides or other non-steroidal modulators of ecdysteroid receptors or insect juvenile hormone receptors. Additionally, because the EcR partner protein (USP polypeptide) subunits of insect steroid receptors, and the USP polypeptides of insect juvenile hormone receptors, bind insect juvenile hormones, a sesquiterpenoid group of ligands that regulate developmental transitions in insects (see Jones and Sharp, 1997), compounds which interfere with the binding of juvenile hormone are also candidate insecticides.

- A further aspect of the present invention provides a method of identifying a modulator of insect steroid receptor-mediated gene expression or insect juvenile hormone receptor-mediated gene

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expression comprising:

- (i) assaying the expression of a reporter gene in the presence of a recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention and a potential modulator; and
- 5 (ii) assaying the expression of a reporter gene in the presence of a recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention and without said potential modulator; and
- (ii) comparing expression of the reporter gene in the presence of the potential modulator to the expression of a reporter gene in the absence of the potential
- 10 modulator,

wherein said reporter gene is placed operably under the control of a steroid response element (SRE) to which said insect steroid receptor binds or a promoter sequence comprising said SRE.

In the present context, a "modulator" is a compound or molecule that agonises or antagonises the binding properties and/or biological activity of a receptor polypeptide or holoreceptor. Preferred modulators according to this embodiment include those synthetic compounds that are suitable for use as insecticidally-active agents described *supra*.

The reporter gene may be any gene, the expression of which may be monitored or assayed readily. Preferably, the reporter gene is a structural gene that encodes a peptide, polypeptide or enzyme that is assayed readily by enzymic or immunological means, for example the  $\beta$ -galactosidase,  $\beta$ -glucuronidase, luciferase or chloramphenicol acetyltransferase (CAT) genes. Alternatively, the reporter gene may be a gene which encodes an immunologically-detectable protein, for example a FLAG peptide, poly-lysine peptide or poly-histidine peptide.

25

Standard methods are used to assay the expression of the reporter gene.

This embodiment of the invention may be applied directly to the identification of potential insecticidally-active compounds or alternatively, modified for such purposes by assaying for the binding (direct or indirect) of the recombinant or isolated insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide of the invention to a steroid response element (SRE),

30

rather than by assaying for reporter gene expression. According to this alternative embodiment, the binding assayed in the presence or absence of a potential insecticidally-active compound is compared, wherein a difference in the level of binding indicates that the candidate compound possesses potential insecticidal activity.

5

In addition, substances may be screened for insecticidal activity by assessing their ability to bind, *in vivo* or *in vitro*, to the intact ecdysone receptor or alternatively, the ligand-binding regions of the EcR polypeptide subunit of the ecdysone receptor (eg. SEQ ID NO: 2 or SEQ ID NO: 10 or SEQ ID NO: 14) or the EcR partner protein ( USP polypeptide) of the ecdysone  
10 receptor (eg. SEQ ID NO: 4 or SEQ ID NO: 6 or SEQ ID NO: 8 or SEQ ID NO: 16 or SEQ ID NO: 18 or SEQ ID NO: 20 or SEQ ID NO: 22 or SEQ ID NO: 38 or SEQ ID NO: 40 or SEQ ID NO: 42). Competition assays involving the native insect steroid may be employed to assess insecticidal activity.

15 The performance of this embodiment may, for example, involve binding the insect steroid receptor polypeptide to a support such as a plurality of polymeric pins, whereafter the polypeptide resident on the plurality of pins is brought into contact with candidate insecticidal molecules for screening. The molecules being screened may be isotopically labelled so as to permit ready detection of binding. Alternatively, reporter molecules may be utilized which bind  
20 to the insect steroid receptor candidate molecule complex. Alternatively, compounds for screening may be bound to a solid support, such as a plurality of pins which are then reacted with the thermostable insect steroid receptor or complex with a partner protein. Binding may, for example, be determined again by isotopic-labelling of the receptor, or by antibody detection or use of another reporting agent.

25

In an alternative embodiment, insecticidally-active agent are identified using rational drug design, by expressing a USP polypeptide of a juvenile hormone receptor or a fragment thereof which includes the ligand-binding region, optionally in association with an EcR polypeptide of a steroid receptor or ligand binding domain thereof, and optionally in association with an insect  
30 steroid or analogue thereof, so as to form a complex, determining the three-dimensional structure of the ligand binding domain of the complex, and identifying compounds which bind

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to or associate with the three-dimensional structure of the ligand binding domain, wherein said compounds represent candidate insecticidally-active agents.

The methods described herein for identifying modulators of gene expression and insecticidal compounds, may be performed using prokaryotic or eukaryotic cells, cell lysates or aqueous solutions.

A further aspect of this invention accordingly relates to synthetic compounds derived from the three dimensional structure of EcR polypeptides or EcR partner protein (USP polypeptide) subunits of insect steroid receptors, or fragments thereof, or insect steroid receptors or fragments thereof, or USP polypeptides of insect juvenile hormone receptors or fragments thereof, which compounds are capable of binding to said receptors which have the effects of either inactivating the receptors (and thus acting as antagonists) or potentiating the activity of the receptor.

15 By "derived from" it is meant that the compounds are based on the three dimensional structure of the aforementioned proteins, that is, synthesized to bind, associate or interfere with insect steroid binding or juvenile hormone binding.

20 The compounds may bind strongly or irreversibly to the ligand binding site or another region of  
the receptor or USP and act as agonists or antagonists of insect steroids, or juvenile hormone  
binding, or otherwise interfere with the binding of ligand, such that ecdysteroids or juvenile  
hormones. Such compounds would have potent insecticidal activity given the key role of insect  
steroids, or juvenile hormone, in insect physiology and biochemistry. Such compounds would  
25 also possess a unique specificity.

This invention is also described with reference to the following non-limiting examples.

### EXAMPLE 1

#### **Construction of a plasmid (pSV40-EcR) expressing the EcR polypeptide subunit of the *D. melanogaster* ecdysone receptor**

A 3110 base-pair *FspI-HindIII* fragment was excised from a cDNA encoding the EcR polypeptide subunit of the *D. melanogaster* ecdysone receptor (Koelle *et al.*, 1991), the excised sequence comprising the complete 2634 base pair coding region and 214 base pairs of 5'-leader sequence and 258 base pairs of 3'- untranslated sequence. The fragment was ligated into the *Bam*HI site of the expression plasmid pSG5 (Greene *et al.*, 1988) to produce the expression plasmid pSV40-EcR, wherein expression of the EcR polypeptide subunit of the *Drosophila melanogaster* ecdysone receptor is placed operably under the control of the SV40 promoter sequence.

### EXAMPLE 2

#### **Construction of the reporter plasmid p(EcRE)<sub>7</sub>-CAT**

The reporter plasmid p(EcRE)<sub>7</sub>-CAT was constructed by insertion of multiple copies (i.e. 5 to 7 copies) of the hsp27 ecdysone response element, containing a central 13 base pair palindromic ecdysone response element (EcRE), derived from the hsp27 gene (Riddihough and Pelham, 1987) into the *HindIII* site of the plasmid pMMTV-CAT (Hollenberg and Evans, 1988), 93 base pairs upstream of the transcription start site of the MMTV promoter, thereby operably connecting expression of the chloramphenicol acetyltransferase structural gene to regulation by an insect receptor which binds to the hsp27 ecdysone response element.

### EXAMPLE 3

#### **Cell Culture and Transient Transfection**

Chinese hamster ovary (CHO) cells were maintained in 50% (v/v) Dubbecco's modified Eagle's medium (DMEM) and 50% (v/v) Hamm F12 nutrient mixture (GIBCO) supplemented with 10% (v/v) foetal bovine serum. Transfection was carried out by the DNA-calcium phosphate co-precipitation method (Ausubel *et al.*, 1992). One day before transfection with the plasmids described in Examples 1 and/or 2, or other expression plasmids, CHO cells were plated out at 5 - 8 x 10<sup>5</sup> cells per 6 cm diameter culture dish in the above DMEM/F12 medium. Three

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hours before the addition of the DNA-calcium phosphate co-precipitate, the cells were washed with phosphate buffered saline (PBS; Sambrook *et al.*, 1989) and cultured in fresh DMEM plus 10% (v/v) foetal bovine serum. The cells were incubated in the presence of the co-precipitate for eighteen hours before excess DNA was removed by washing with PBS. The cells were then  
5 cultured for another day in DMEM/F12 supplemented with 10% (v/v) foetal bovine serum with or without added ponasterone A (PNA), before harvesting. Cells were washed with PBS, harvested by mechanical scraping in 0.25 M Tris-HCl (pH 7.8), and disrupted by three freeze-thaw cycles.

10 All transfections included, in addition to expression and reporter plasmids, a  $\beta$ -galactosidase-expressing plasmid designated pPgK-LacZ (McBurney *et al.*, 1991), which served as an internal control for the efficiency of transfection, and pUC18 DNA in an amount sufficient to produce 10  $\mu$ g total DNA per culture dish.

15 The chloramphenicol acetyltransferase (CAT) and  $\beta$ -galactosidase activities encoded by the reporter genes present in the reporter plasmids were assayed as described in Sambrook *et al.*, (1989). Cells that were co-transfected with p(EcRE)<sub>7</sub>-CAT and pSV40-EcR clearly showed induction of CAT activity in the presence of PNA, showing 50 units of activity. Controls showed negligible activity.

20

We have observed that the ecdysone receptor can lead to stimulation of expression from an ecdysone responsive promoter in some cell types, for example in CHO cells, but not in CV-1 cells. Whilst not being bound by any theory or mode of action, this may reflect a cell-type specific distribution of at least one other transcription factor essential for ecdysone  
25 responsiveness. To determine cell types suitable for expressing reporter genes under the control of the steroid receptor of the present invention, the cell-type specificity of ecdysone-responsive gene expression is assayed in cell-free transcription lysates derived from several target cell lines. Additionally, by fractionating or isolating the nuclear proteins of cell lines that express the reporter genes and supplementing lysates derived from non-expressing cell lines  
30 with such nuclear protein fractions or isolated proteins, any essential auxiliary factors are defined and the genes encoding them cloned. Co-transfection of the receptor-encoding genes

30



TABLE 1

pSV40-EcR ( $\mu$ g/dish)	PNA ( $\mu$ M)	Fold-induction of expression	
		37°C	30°C
2.5	20	14X	35X
	100	59X	54X
0.5	20	8X	26X
	100	47X	33X
0.1	20	1.6X	25X
	100	9.0X	39X

## EXAMPLE 6

10                    **Cloning and characterization of a cDNA molecule encoding  
the EcR polypeptide of the *L. cuprina* ecdysone receptor**

Rationale for amplification primer design

The nucleotide sequences of the primers Rdna3 (SEQ ID NO: 23) and Rdna4 (SEQ ID NO: 24) were derived from the amino acid sequence conserved between the DNA-binding domains of  
15 the EcR polypeptide subunits of the *D. melanogaster* and *C. tentans* ecdysone receptors. However, amino acid sequences homologous to two other members of the steroid receptor superfamily of *D. melanogaster*, *Drosophila* hormone-receptor 3 (DHR3; Koelle, *et al.*, 1991) and *Drosophila* early gene (E75; Segraves and Hogness, 1990) were excluded from the primer designs, to reduce the possibility of amplifying the *L. cuprina* homologues of genes encoding  
20 DHR3 or E75 by PCR.

Amplification primers and PCR conditions

A 105 base pair DNA fragment, encoding the DNA-binding domain of the EcR polypeptide subunit of the *L. cuprina* ecdysone receptor, was amplified from the *L. cuprina* genome by PCR,  
25 by using the following degenerate primers:

Rdna3 (32mer with EcoRI site):

5'-CGGAATTCCGCCTCTGGTTA(C/T)CA(C/T)TA(C/T)AA(C/T)GC 3' (i.e. SEQ ID NO: 23);

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and

Rdna4 (32mer with BamHI site):

5'-CGCGGATCC(G/A)CACTCCTGACACTTTCG(C/T)CTCA 3' (i.e. SEQ ID NO: 24).

- 5 Amplification reactions employed *TaqI* DNA polymerase (Promega) and the following amplification conditions:  
 cycle 1: 97°C/5 minutes, 50°C hold; add polymerase 50°C/5 minutes;  
 cycles 2-3: 72°C/3 minutes, 94°C/1 minute, 50°C/1 minute;  
 cycles 4-43: 72°C/3 minutes, 94°C/1 minute, 55°C/1 minute;  
 10 cycle 44: 72°C/10 minutes.

- To facilitate cloning of the amplified fragments for use as hybridisation probes, the 5' end of primer Rdna3 contained an *EcoRI* site and the 5' end of primer Rdna4 contained a *BamHI* site. The amplified *L. cuprina* gene fragments were cloned into *pBluescript SK+*, following digestion
- 15 using the enzymes *EcoRI* and *BamHI*, purification of the digested DNA by agarose gel electrophoresis and electro elution of the product band.

#### Hybridisation probe preparation

- For probe preparation, the insert was cut out of the *pBluescript SK+* vector using *EcoRI* and
- 20 *BamHI*, and <sup>32</sup>P-labelled using the GIGAprime DNA Labelling Kit (Bresatec Limited, Adelaide, Australia) essentially according to the manufacturer's instructions, except that random primers were replaced with the specific primers Rdna3 and Rdna4 (see above). Unincorporated label was removed by size exclusion chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used at 10<sup>6</sup> cpm/ml in hybridizations.

25

#### Construction and screening of *L. cuprina* cDNA libraries

- Two independent *L. cuprina* cDNA libraries derived from late third instar *L. cuprina* larvae were prepared by random priming and oligo-dT priming respectively, and cloned into the *EcoRI* site of the *Lambda/ZapII* vector (Stratagene). The primary libraries generated were subsequently
- 30 amplified according to the manufacturer's instructions, using standard protocols.

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Both cDNA libraries generated are superior to existing *L. cuprina* libraries in terms of their phage titre (i.e. pfu/ml) and insert sizes (0.5 - 4 kbp in both cases). In particular, the primary oligo-dT primed library comprised  $4.7 \times 10^6$  pfu, whilst the amplified oligo-dT primed library comprised  $7.5 \times 10^{10}$  pfu/ml; the primary random-primed library comprised  $1.3 \times 10^6$  pfu, whilst  
 5 the amplified random-primed library comprised  $3.4 \times 10^{10}$  pfu/ml.

The prepared cDNA libraries were screened by lifting 500,000 plaques from each library in duplicate on to Hybond N membranes (Amersham) and hybridizing same under low stringency conditions to the  $^{32}\text{P}$ -labelled amplification product produced using the primers Rdna3 and  
 10 Rdna4 (see above). In particular, hybridisations were performed for twenty four hours at  $37^\circ\text{C}$  in a hybridisation solution comprising 42% (w/v) formamide; 5 x SSPE solution; 5 x Denhardt's solution; and 0.1% (w/v) sodium dodecyl sulphate, as described essentially by Ausubel *et al.*, (1992) or Sambrook *et al.* (1989). The membranes were then washed at  $37^\circ\text{C}$  in 2XSSC solution containing 0.1% (w/v) sodium dodecyl sulphate. Following washing, positive plaques  
 15 were detected by autoradiography, using XOMAT-AR film (Kodak) for two to three days, at -  $70^\circ\text{C}$ .

Two positive-hybridising plaques were obtained from screening of the random-primed library (containing cDNA inserts comprising 561 base pairs and 1600 base pairs in length,  
 20 respectively), and one positive-hybridising plaque was obtained from the screening of the oligo-dT primed library (containing a cDNA insert comprising approximately 3400 base pairs in length). pBluescript phagemids containing cDNA inserts were excised *in vivo* from these positive plaques using the Exassist Helper Phage system (Stratagene).

25 The nucleotide sequences of the isolated cDNA clones were obtained using the USB Sequenase Version 2.5 Kit. Sequence data obtained indicated that the 561 bp and 1600 bp cDNAs encode amino acid sequences comprising the important DNA-binding domain and the hormone-binding domain of the EcR polypeptide subunit of the *L. cuprina* ecdysone receptor, whilst the 3400 bp cDNA comprises an entire 2274 bp open reading frame encoding the EcR  
 30 polypeptide subunit of the *L. cuprina* ecdysone receptor. Accordingly, the 3400 bp cDNA is a full-length cDNA clone. The nucleotide sequence of the open reading frame and 3'-

25 For *M. persicae* probe preparation, the amplified 105 bp DNA insert was excised from the *p*Bluescript SK+ vector using EcoRI and BamHI, and <sup>32</sup>P-labelled using the GIGAprime DNA Labelling Kit (BresaGen Limited, Adelaide, Australia) essentially according to the manufacturer's instructions, except that random primers were replaced with the specific primers Mdna1 and Mdna2 (see above). Unincorporated label was removed by size exclusion  
30 chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used at 10<sup>6</sup> cpm/ml in hybridizations.

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Construction and screening of *M. persicae* cDNA libraries:

Two independent *M. persicae* cDNA libraries derived from late third instar *M. persicae* larvae were prepared by random priming and oligo-dT priming respectively, and cloned into the *EcoRI* site of the *Lambda/ZapII* vector (Stratagene). The primary libraries generated were  
5 subsequently amplified according to the manufacturer's instructions, using standard protocols.

Both cDNA libraries generated are superior to existing *M. persicae* libraries in terms of their phage titre (i.e. pfu/ml) and insert sizes (0.5 - 4 kbp in both cases). In particular, the primary oligo-dT-primed library comprised  $1 \times 10^7$  pfu, whilst the amplified oligo-dT primed library  
10 comprised  $1 \times 10^{10}$  pfu/ml; the primary random-primed library comprised  $1 \times 10^6$  pfu, whilst the amplified random-primed library comprised  $2 \times 10^{11}$  pfu/ml.

Additionally, a further cDNA library was produced in the Lambda ZAP Express insertion vector (Stratagene). To produce this library, cDNA derived from late third instar *M. persicae* larvae was  
15 prepared by oligo-dT priming and cloned directionally into *EcoRI*-*XhoI* digested vector DNA. The primary library comprised  $1 \times 10^6$  pfu, whilst the amplified oligo-dT primed library comprised  $1 \times 10^9$  pfu/ml, with insert sizes in the range 0.5 - >4 kbp.

The random-primed *M. persicae* cDNA phage library was screened as described in Example  
20 6, using the *M. persicae* hybridisation probe prepared as described above.

A single positive-hybridising plaque was isolated and sequenced according to standard procedures. The nucleotide sequence of this clone is set forth herein as SEQ ID NO: 9. This cDNA clone comprises a 585bp protein-encoding sequence which encodes the DNA-binding  
25 domain of a EcR polypeptide of a putative *M. persicae* ecdysone receptor. The amino acid sequence encoded by this partial cDNA clone is set forth herein as SEQ ID NO: 6.

**EXAMPLE 8****Second attempt at cloning and characterization of a cDNA molecule encoding the EcR polypeptide of the *M. persicae* ecdysone receptor**Hybridisation probe preparation

- 5 Further hybridisation probes specific for the EcR polypeptide of the *M. persicae* ecdysone receptor were generated using PCR from the Lambda ZAPII oligo dT-primed library using primers AP1 and AP2. The forward primer AP1 was designed to anneal to nucleotide sequences of the partial cDNA (SEQ ID NO: 9) encoding part of the first zinc finger motif present in the DNA-binding domain. The reverse primer, AP2, was adapted from degenerate
- 10 primers designed to anneal to nucleotide sequences complementary to those encoding an EcR ligand binding domain (Kamimura *et al.*, 1996). The nucleotide sequences of primers AP1 and AP2 are as follows:

- Primer AP1: 5'- TCGTCCGGTTACCATTACAACGC -3' (SEQ ID NO: 27); and
- 15 Primer AP2: 5'- TAGACCTTTGGC(A/G)AA(C/T)TC(A/G/C/T)ACAAT -3'(SEQ ID NO: 28)

- The PCR reaction mixture contained 4 µl of each primer (50 pm/µl), 5 µl of deoxynucleotide triphosphate mix (2mM), 1 µl of aphid oligo dT primed Lambda ZAPII cDNA library, 1 µl of recombinant *Pfu* DNA Polymerase (5 units/µl, Stratagene®), 5 µl of 10x *Pfu* buffer
- 20 (Stratagene®) and 30 µl of MilliQ water. The *Pfu* polymerase was used in this reaction because it possesses proof-reading activity, which reduces the possibility of misincorporation of nucleotides. The PCR conditions included 42 cycles, each cycle comprising annealing at 55°C, extension at 72°C and melting at 94°C.

- 25 The major amplification product obtained in this reaction was gel-purified, kinased and ligated into the *Sma*I site of pUC18.

- To screen *M. persicae* cDNA libraries, the cloned amplification product was digested to generate two non-overlapping probes, designated "EcR probe 1" (i.e. SEQ ID NO: 11) and
- 30 "EcR probe 2" (i.e. SEQ ID NO: 12). In this regard, digestion of the cloned product with *Sph*I produced a DNA fragment comprising a nucleotide sequence specific for a region encoding the

DNA-binding domain (EcR probe 1; SEQ ID NO: 11), whilst digestion with *SphI/EcoRI* produced a DNA fragment comprising a nucleotide sequence having homology to a region encoding a putative linker domain, designated domain D, and the 5'-end of a putative hormone-binding domain, present in the EcR polypeptide of the insect ecdysone receptors (EcR probe 2, SEQ ID NO: 12).

EcR probe 1 and EcR probe 2 were labelled with [ $\alpha$ -<sup>32</sup>P]dATP in a reaction catalysed by Klenow fragment. All reagents were components of a GIGAprime DNA labelling kit (BresaGen Limited, Adelaide, Australia), except that the random primers were replaced with specific oligonucleotides synthesised to be complementary to the ends of EcR probe 1 and EcR probe 2.

#### Screening of *M. persicae* cDNA libraries

480,000 plaques from the oligo dT primed Lambda Zap Express cDNA library (Example 7) were screened as described above, using EcR probe 1. This approach yielded about 300 positive clones. Positive-hybridising clones were pooled and rescreened separately using EcR probe 1 and EcR probe 2, on duplicate lifts. Only four plaques were identified which hybridised to both probes. One of these was found by sequencing to contain a full-length cDNA encoding the EcR polypeptide of the *M. persicae* ecdysone receptor. The nucleotide sequence of the open reading frame of this cDNA is set forth herein as SEQ ID NO: 9. The derived amino acid sequence of the EcR polypeptide subunit of the *M. persicae* ecdysone receptor encoded by this open reading frame is set out in SEQ ID NO: 10.

25

### EXAMPLE 9

#### ***In vivo* function of recombinant EcR polypeptides of the *L. cuprina* ecdysone receptor**

##### Construction of plasmid pF3

Plasmid pF3 was constructed in four steps as follows:

First, plasmid p5S1, comprising the full-length cDNA encoding the EcR polypeptide of the *L. cuprina* ecdysone receptor, was digested with *EatI* and a 3' *EatI* cDNA fragment thus

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generated, encoding the C-terminal end of the EcR polypeptide of the *L. cuprina* ecdysone receptor, was end-filled and sub-cloned into the HindIII site of pUC19, to construct plasmid pEAR. In plasmid pEAR, the 3' end of the cDNA was oriented towards the KpnI site of the pUC19 vector.

5

Second, plasmid p5S1 was also digested separately with:

- (1) *ApoI* and *PstI*, to isolate the 5' end of the cDNA as a 179 bp fragment (fragment A);
- (2) *PstI* and *SpeI*, to isolate a 1650 bp cDNA fragment (fragment B); and
- (3) *SpeI* and *BglII*, to isolate a 203 bp fragment (fragment C).

10

Third, plasmid pEAR was digested with *BglII* and *KpnI*, to isolate the 3' end of the cloned cDNA fragment therein as a 313 bp fragment (fragment D).

Fourth, DNA fragments A, B, C and D were each isolated by agarose electrophoresis and  
15 ligated together into pBluescriptSK+, which had been digested with *EcoRI* and *KpnI*, to produce plasmid pF3.

Plasmid pF3 thus contains the complete open reading frame of the cDNA encoding the EcR polypeptide of the *L. cuprina* ecdysone receptor, as a 2368 bp fragment located between two  
20 *BamHI* sites.

#### Construction of plasmid pSGLcEcR and plasmid pLcK8

Plasmid pSGLcEcR was constructed by cloning the 2368 bp *BamHI* fragment from pF3, into the *BamHI* site of the mammalian expression vector pSG5 (Stratagene). Plasmid pLcK8 is a  
25 clone of pSGLcEcR.

#### Construction of plasmid pSGDmEcR

Plasmid pSGDmEcR is identical to plasmid pSV40-EcR (Example 1) comprising the EcR polypeptide of the *D. melanogaster* ecdysone receptor placed operably under control of the  
30 SV40 promoter.



#### Transfection of CHO cells

CHO cells were co-transfected with a mixture comprising the following DNAs, lysed and assayed for CAT and  $\beta$ -galactosidase enzyme activity, as described in the preceding Examples:

- (1) one of the expression plasmids designated pSGDmEcR, or pSGLcEcR, or the parental expression plasmid pSG5 as a negative control, at a concentration of 1  $\mu$ g/ml; and
  - (2) the CAT reporter plasmid p(EcRE)<sub>7</sub>-CAT at a concentration of 1  $\mu$ g/ml; and
  - (3) an independent LacZ reporter plasmid, pPGKLacZ, at a concentration of 1  $\mu$ g/ml, included as a control to monitor transfection efficiency .
- 10 CAT reporter gene expression was induced with 10  $\mu$ M or 50  $\mu$ M Muristerone A. In control samples, cells received only the carrier ethanol in place of Muristerone A.

ELISA was used to quantify the synthesis of CAT and  $\beta$ -galactosidase enzymes, in extracts of cells forty eight hours after transfection. Account was taken of the variation between experiments, by normalizing the level of CAT enzyme to the level of  $\beta$ -galactosidase enzyme present in the same extract. Fold induction represents the normalized values for CAT gene expression in cells transfected with pSGDmEcR, pSGLcEcR or pSG5 in the presence of hormone divided by the normalized values for CAT gene expression in cells transfected with the same plasmid but in the absence of hormone. The average values of three independent experiments are shown in Figure 1 and the error bars indicate standard error of the mean.

Data shown in Figure 1 indicate that the EcR polypeptide of the *L. cuprina* ecdysone receptor from Example 3 is biologically active *in vivo*. CAT induction is observed at both 50  $\mu$ M and 10  $\mu$ M steroid (Muristerone A), with about 30 and 15 fold induction respectively. In view of the *in vivo* activity of the EcR polypeptide of the *L. cuprina* ecdysone receptor obtained according to this protocol, potential insecticidal substances acting by interaction with an insect steroid receptor, such as an ecdysone receptor, are screened by addition of the substances to the *in vivo* assay described herein. Substances are added in an amount from 0.05  $\mu$ M to 100  $\mu$ M. Candidate insecticidal compounds are identified by their ability to modulate the reporter gene expression which results from trans-activation by the EcR polypeptide of the *L. cuprina* ecdysone receptor.

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**EXAMPLE 10****Chimeric EcR polypeptides of insect ecdysone receptors**

Chimeric ecdysone receptors comprising regions derived from EcR polypeptides of ecdysone receptors of different species are produced and assayed for enhanced activity. In a particularly preferred embodiment, a chimeric ecdysone receptor is produced using the EcR polypeptides of the *D. melanogaster*, *M. persicae* and *L. cuprina* ecdysone receptors.

In one exemplification of this embodiment, plasmids pSGLD and pSGDL are produced comprising coding regions derived from the EcR polypeptides of the *D. melanogaster* and *L. cuprina* ecdysone receptors. In plasmid pSGLD, the 5'-end of the open reading frame of the *D. melanogaster* sequence, encoding the N-terminal portion of the EcR polypeptide of the *D. melanogaster* ecdysone receptor to the end of the DNA-binding domain of said polypeptide, is fused to the 3'-end of the open reading frame of the *L. cuprina* sequence, encoding the C-terminal portion of the EcR polypeptide of the *L. cuprina* ecdysone receptor, from the D domain and hormone-binding domain to the carboxyl terminus. In plasmid pSGDL, the 5'-end of the open reading frame of the *L. cuprina* sequence, encoding the N-terminal portion of the EcR polypeptide of the *L. cuprina* ecdysone receptor to the end of the DNA-binding domain of said polypeptide, is fused to the 3'-end of the open reading frame of the *D. melanogaster* sequence, encoding the C-terminal portion of the EcR polypeptide of the *D. melanogaster* ecdysone receptor, from the D domain and hormone-binding domain to the carboxyl terminus. These plasmids thus encode chimeric EcR polypeptides which form ecdysone receptor variants.

As shown in Figure 2, chimeric EcR polypeptides of *L. cuprina* and *D. melanogaster* ecdysone receptors, comprising fusion polypeptides between the DNA-binding domains and hormone-binding domains of the base *L. cuprina* and *D. melanogaster* polypeptides, exhibit bioactivity when measured in the CAT assay described above. Significant bioactivity of the chimeric EcR polypeptides encoded by plasmids pSGLD and pSGDL, comparable to the bioactivity of the *D. melanogaster* base EcR polypeptide, is observed at both 10  $\mu$ M and 50  $\mu$ M concentrations of Muristerone A.

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**EXAMPLE 11****Isolation and characterisation of a full-length cDNA encoding the EcR partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor**

The EcR partner protein (USP polypeptide) subunit of the *L. cuprina* ecdysone receptor also  
 5 functions alone as a USP polypeptide of the *L. cuprina* juvenile hormone receptor. A cDNA encoding both receptor polypeptide activities was isolated using PCR and hybridisation as follows.

Hybridisation probe preparation

10 A 150 base-pair probe, specific for genetic sequences encoding the EcR partner protein (USP polypeptide) subunit of insect ecdysone receptors or the USP polypeptide subunit of insect juvenile hormone receptors (SEQ ID NO: 21), was isolated by PCR from *L. cuprina* genomic DNA using the degenerate primers described by Tzertzinis *et al.* (1994). The PCR reaction conditions were as described in Example 6, except that *Pfu* polymerase was used in place of  
 15 *TaqI* polymerase.

The amplified DNA fragment was sub-cloned into *EcoRI* and *ClaI* double-digested *pBluescript* SK+ vector (Stratagene), after double-digestion of the fragment using the enzymes *EcoRI* and *ClaI*, purification of the amplified fragment by agarose gel electrophoresis, and electro elution  
 20 of the product band. The nucleotide sequence of the probe was obtained using the USB Sequenase version 2.0 Kit ( SEQ ID NO: 21).

For probe preparation, the amplified *L. cuprina* DNA fragment was excised from the vector using *EcoRI* and *SalI*, gel purified and <sup>32</sup>P-labelled using the GIGAprime DNA Labelling Kit  
 25 (BresaGen Limited, Adelaide, Australia) essentially according to the manufacturer's instructions except that random primers were replaced with the two degenerate primers described by Tzertzinis *et al.* (1994) (see above). Unincorporated label was removed by size exclusion chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used at 10<sup>6</sup> cpm/ml in hybridizations.

30

Screening of *L. cuprina* cDNA libraries

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The *L. cuprina* cDNA library described above (Example 6) was screened with the amplified probe as described in Example 6. From one positive plaque, we derived plasmid pLSP4 containing a 3800 bp insert. Sequencing revealed that the 5' portion of pLSP4 encodes the EcR partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor, followed by a long (2.4 kb), apparently untranslated region (UTR). A 2453 bp *EcoRI* fragment of plasmid pLSP4 was isolated and sub-cloned into pBluescript SK+ (Stratagene), to construct plasmid pBLU1, which contains the full-length cDNA sequence. The nucleotide sequence of the full-length cDNA present in pBLU1 and the amino acid sequence encoded therefor, are set forth herein as SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

10

The open reading frame (ORF) of SEQ ID NO: 3 encodes a polypeptide comprising 467 amino acids in length. The ATG start codon is located within a very favourable translational start context (i.e. 5'-GAAAATG-3') having 75% identity to the consensus sequence (i.e. 5'-C/AAAAATG-3') for *D. melanogaster* mRNA sequences (Cavener *et al.*, 1987). Moreover, the derived amino acid sequence of the *L. cuprina* EcR partner protein (USP polypeptide) comprises domains A/B, C, D, and E/F that are characteristic of nuclear hormone receptors (Evans, 1988; Forman and Samuels, 1990).

The nucleotide sequences of the 5'- untranslated region and coding region of the cDNA contained in plasmid pLSP5, and the amino acid sequence encoded therefor, are set forth herein as SEQ ID NO: 5 and SEQ ID NO: 6, respectively. The nucleotide sequences of the 5'- untranslated region and coding region of the cDNA contained in plasmid pLSP12, and the amino acid sequence encoded therefor, are set forth herein as SEQ ID NO: 7 and SEQ ID NO: 8, respectively.

25

Nucleotide sequence analyses revealed differences in the 5'- untranslated regions of pLSP4, pLSP5, and pLSP12, however the coding regions appeared to be identical, suggesting a possible splice variation. This conclusion is supported by the fact that the cDNAs of pLSP4, pLSP5 and pLSP12 contained identical nucleotide sequences within their 5'- untranslated regions, however differed by the addition/deletion of sequences. In particular, the 5'-terminal 13 nucleotides of all three cDNA clones were identical, as was the nucleotide sequence

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surrounding the translation start codon (i.e. 5'-AAAATG-3'). Clone pLSP5 (SEQ ID NO: 5) differed from clone pLSP 4 (SEQ ID NO: 3) in so far as it included an additional 176 bp of 5'- untranslated sequence inserted between nucleotides 13 and 14 of pLSP4. Clone pLSP12 (SEQ ID NO: 7) also differed from pLSP4 (SEQ ID NO: 3) in so far as it included an additional 116 bp of 5'- untranslated sequence inserted between nucleotides 13 and 14 of pLSP4. Clones pLSP5 (SEQ ID NO: 5) and pLSP12 (SEQ ID NO: 7) differed in so far as pLSP5 included an additional 60 bp of 5'- untranslated sequence inserted between nucleotides 13 and 14 of pLSP12.

- 10 The ATG start codons of both clones pLSP5 and pLSP12 are within translational start context sequences (i.e. 5'-CAAAATG-3') having absolute identity to the consensus sequence (i.e. 5'-C/AAAATG -3') for *D. melanogaster* mRNA sequences (Cavener *et al.*, 1987).

#### EXAMPLE 12

- 15 **Isolation and characterisation of a partial cDNA encoding the EcR partner protein (USP polypeptide) of the *M. persicae* ecdysone receptor**

The EcR partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor also functions alone as a USP polypeptide of the *M. persicae* juvenile hormone receptor. To isolate a partial cDNA encoding both receptor polypeptide activities, a 140 bp probe was amplified from *M. persicae* genomic DNA, by PCR, using the two degenerate primers described by Tzertzinis *et al.* (1994) (see preceding Example). The PCR reaction conditions were as described in Example 6, except that *Pfu* polymerase was used in place of *TaqI* polymerase.

25 The amplified DNA fragment was sub-cloned into *EcoRI* and *ClaI* double-digested *pBluescript* SK+ vector (Stratagene), after double-digestion of the fragment using the enzymes *EcoRI* and *ClaI*, purification of the amplified fragment by agarose gel electrophoresis, and electro elution of the product band.

30 The nucleotide sequence of the insert in the *pBluescript* SK+ vector was obtained using automated fluorescent dye terminator sequencing (SUPAMAC, Sydney Australia).

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#### Hybridisation probe preparation and library screening

For probe preparation the amplified *M. persicae* DNA insert was cut out of the pBluescript+ vector with *EcoRI* and *SalI*, gel purified and <sup>32</sup>P-labelled using the GIGAprime DNA Labelling Kit (Bresatec Limited, Adelaide, Australia) essentially according to the manufacturer's instructions except that random primers were replaced with the degenerate primers described by Tzertzinis *et al.* (1994) (see preceding Example). Unincorporated label was removed by size exclusion chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used at 10<sup>6</sup> cpm/ml in hybridizations to screen the *M. persicae* cDNA library as described in Examples 7 and 8.

10

The positive-hybridising clones were plaque-purified and sequenced using standard procedures as described herein. The nucleotide sequence of the open reading frame of the full-length cDNA encoding the partner protein (USP polypeptide) subunit of the *M. persicae* ecdysone receptor or the USP polypeptide of the *M. persicae* juvenile hormone receptor is set forth herein as SEQ ID NO: 15. The derived amino acid sequence of this open reading frame is set forth as SEQ ID NO: 16.

#### **EXAMPLE 13**

**A construct for the baculovirus-directed co-expression of functional ligand-binding regions of the EcR polypeptide and partner protein (USP polypeptide) of the *D. melanogaster* ecdysone receptor**

A vector was prepared to facilitate the baculovirus-directed co-expression of ligand-binding regions derived from the EcR polypeptide and partner protein (USP polypeptide) of the *D. melanogaster* ecdysone receptor, the protein products of which associate on co-expression to form a functional hormone-binding complex. The associated proteins are then used in high through-put assays or three-dimensional structural analysis. We have found that the ligand-binding domain, together with most of the linker domain of the EcR polypeptide subunit and of the EcR partner protein (USP polypeptide), are sufficient to associate to form a functional hormone-binding complex.

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1. Isolation of the ligand-binding region of the EcR polypeptide of the *D. melanogaster* ecdysone receptor.

A *Sac* I - *Hind* III fragment encoding most of the linker (domain D) and all of the ligand-binding domain (domains E and F) of the EcR polypeptide of the *Drosophila melanogaster* ecdysone  
5 receptor was excised from a plasmid comprising DNA encoding the complete EcR polypeptide (Koelle *et al.* 1991). The excised fragment was cloned into *Sac* I - *Hind* III-digested expression vector pQE31(Qiagen), to produce the plasmid vector pQE31DmECR.

2. Construction of a baculovirus expressing the ligand-binding regions of EcR and USP polypeptides

A baculovirus was constructed for the co-expression in insect cells of:

- (i) a cDNA region comprising a nucleotide sequence which encodes at least the ligand-binding domain and much of the linker domain of the EcR polypeptide of the *D. melanogaster* ecdysone receptor isolated as described at paragraph (1) above; and
- 15 (ii) a cDNA region comprising a nucleotide sequence which encodes at least the ligand-binding domain and much of the linker domain of the partner protein (USP polypeptide) of the *D. melanogaster* ecdysone receptor.

To produce this baculovirus, a *Eco* R I - *Hind* III fragment was excised from pQE31DmECR, said  
20 fragment encoding an oligo-His tag, and most of the linker domain, together with all of the ligand-binding domain of EcR polypeptide. This *Eco* R I - *Hind* III fragment was ligated into *Eco* R I - *Hind* III cleaved pFastBacDUAL, to produce the plasmid pDmEcR.DUAL. To insert gene sequences specific for the partner protein (USP polypeptide), the *Hind* II - *Nsi* I fragment encoding most of the linker and all of the ligand-binding domain of the partner protein (USP  
25 polypeptide) was excised from a full-length cDNA clone in plasmid pZ7-1 (supplied by Vince Henrich) and ligated into *Nco* I - *Nsi* I cleaved pDmEcR.DUAL. A nucleotide sequence encoding a "FLAG" peptide was subsequently incorporated upstream of, and in the same reading frame as, the nucleotide sequence encoding ligand-binding region of the partner protein (USP polypeptide), by ligation into the unique *Sma* I site, thereby producing the plasmid  
30 pDmEcR.USP.DUAL. Plasmids containing the FLAG-encoding nucleotide sequence in the correct orientation were selected by nucleotide sequence determination.

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The segment of pDmEcR.USP.DUAL which encodes the tagged ligand-binding region of the EcR polypeptide and partner protein (USP polypeptide) sequences, placed operably under the control of polyhedrin and p10 promoters, respectively, was recombined into a baculovirus genome, by employing the Tn7 transposition system (Luckow *et al*, (1993). The polypeptide products were then co-expressed in insect Sf21 and Sf9 cells, where they associated into a functional complex.

Expression of the tagged ligand-binding regions of the EcR polypeptide and partner protein (USP polypeptide) sequences was examined by immunoblot analysis of extracts derived from insect Sf21 cells infected with the recombinant baculovirus, employing antibodies directed against the oligo-His and FLAG tags. This analysis detected bands on immunoblot analysis of approximately the predicted sizes for the expressed tagged ligand-binding regions of the EcR polypeptide and partner protein (USP polypeptide).

The protein detected by anti-oligo-His-antibodies was enriched by affinity purification on nickel-NTA resin (Qiagen), and the FLAG-labelled protein was affinity-purified using FLAG M2 Affinity Gel (Kodak). It was further demonstrated that the oligo-His-tagged EcR polypeptide and the FLAG-tagged EcR partner protein (USP polypeptide) bound as a hetero-oligomeric complex to FLAG M2 Affinity Gel (Kodak).

20

Furthermore, binding assays, performed using a modification of the method of Yund *et al* (1978), demonstrated a highly-significant increase in the binding of the a labelled ecdysone analogue, [<sup>3</sup>H] ponasterone A, in cells infected by the recombinant baculovirus, compared to the binding observed for the naturally-occurring ecdysone holoreceptor in *L. cuprina* embryos. In contrast, cells infected by a control virus displayed neither antibody-positive bands on western analysis, nor specific binding of [<sup>3</sup>H] ponasterone A, above background levels. These data indicate correct folding and association of the variant polypeptides comprising the ligand-binding regions of the *D. melanogaster* EcR polypeptide and *D. melanogaster* partner protein (USP polypeptide). The correctly-folded and associated complex formed by the truncated EcR polypeptide and truncated EcR partner protein (USP polypeptide), is used for X-ray and NMR structural analysis and for high-throughput screens.



# EXAMPLE 14

## Construct for the baculovirus-directed co-expression of functional ligand-binding regions of the EcR polypeptide and partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor

5

A vector for the baculovirus-directed co-expression of ligand-binding domains derived from the EcR polypeptide and partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor was prepared essentially as described in the preceding Example.

10

### 1. Isolation of the ligand-binding region of the EcR polypeptide of the *L. cuprina* ecdysone receptor.

A *SphI* – *KpnI* fragment encoding most of the linker (domain D) and all of the ligand-binding domain (domains E and F) of the EcR polypeptide of the *L. cuprina* ecdysone receptor was excised from a cDNA clone encoding the complete EcR polypeptide and cloned into the *SphI* – *KpnI* cleaved expression vector pQE32 (Qiagen), to produce the plasmid pQE32LcEcR.

### 2. Isolation of the ligand-binding region of the partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor.

A DNA fragment encoding most of the linker domain and all of the ligand-binding domain of the partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor was sub-cloned to produce the plasmid pBLU1.

### 3. Construction of a baculovirus expressing the ligand-binding regions of *L. cuprina* EcR and USP polypeptides

A baculovirus was constructed for the co-expression in insect cells of:

- (i) a cDNA region comprising a nucleotide sequence which encodes at least the ligand-binding domain and much of the linker domain of the EcR polypeptide of the *L. cuprina* ecdysone receptor isolated as described at paragraph (1) above; and
- (ii) a cDNA region comprising a nucleotide sequence which encodes at least the

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ligand-binding domain and much of the linker domain of the partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor isolated as described at paragraph (2) above.

- 5 To produce this baculovirus, a *EcoR* I – *Pst*I fragment derived from plasmid pQE32LcEcR, encoding an oligo-His tag and most of the linker domain together with all of the ligand-binding domain of the *L. cuprina* EcR polypeptide was ligated into *EcoR*I– *Pst*I cleaved pFastBac.DUAL, to produce the plasmid pLcEcR.DUAL. An *Av*all–*EcoR*V fragment, encoding most of the linker and all of the ligand-binding domain of *L. cuprina* partner protein (USP  
10 polypeptide) was excised from plasmid pBLU1 and ligated, together with a "FLAG" encoding sequence into the *Pvu*II site of pLcEcR.DUAL, to produce plasmid pLcEcR.USP.DUAL .

The segment of pLcEcR.USP.DUAL which encodes the tagged ligand-binding regions of the EcR polypeptide and partner protein (USP polypeptide) sequences, placed operably under the  
15 control of polyhedrin and p10 promoters, respectively, was recombined into a baculovirus genome, by employing the Tn7 transposition system (Luckow *et al*, (1993). The polypeptide products were then co-expressed in insect Sf21 and Sf9 cells, where they associated into a functional complex.

- 20 Expression was examined by immunoblot analysis. Antibodies directed against oligo-His and FLAG tags detected bands on immunoblot analysis of approximately the predicted sizes for the expressed EcR and USP polypeptide regions respectively, in extracts from insect Sf21 cells infected with the recombinant baculovirus. The protein detected by anti-oligo-His was greatly enriched utilising a nickel-NTA resin (Qiagen) and the FLAG-labelled protein purified on FLAG  
25 M2 Affinity Gel (Kodak). It was also demonstrated by immunoblot analysis that oligo-His-tagged *L. cuprina* truncated EcR polypeptides and FLAG-tagged *L. cuprina* truncated EcR partner protein (USP polypeptide) bind as a hetero-oligomeric complex to FLAG M2 Affinity Gel (Kodak).

- 30 Furthermore, binding assays, carried out by a modification of the method of Yund *et al* (1978), demonstrated a highly-significant increase in the binding of the tritiated ecdysone analogue,

These data indicate correct folding and association of the variant polypeptides comprising the ligand-binding regions of the *L. cuprina* EcR polypeptide and *L. cuprina* partner protein (USP polypeptide). The correctly-folded and associated complex formed by the truncated EcR polypeptide and truncated EcR partner protein (USP polypeptide), is used for X-ray and NMR structural analysis and for high-throughput screens.

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### EXAMPLE 15

#### **A construct for the expression of the ligand-binding region of the USP polypeptide of the *L. cuprina* juvenile hormone receptor**

5 The donor plasmid pLcEcR.USP.DUAL (Example 14) was digested with *Bss*HI and *Pst*I to remove the *L. cuprina* EcR polypeptide-encoding segment therein, thereby leaving the tagged ligand-binding region of the *L. cuprina* USP polypeptide-encoding nucleotide sequence. The digested plasmid was blunt-ended using T4 DNA polymerase and Klenow polymerase, isolated by gel purification, and finally re-ligated to produce the plasmid pLcUSP.SINGLE.

10

To produce recombinant baculovirus capable of expressing the tagged ligand-binding regions of the USP polypeptide, the segment of pLcUSP.SINGLE encoding this polypeptide and the p10 promoter sequence to which said segment is operably connected, was recombined into a baculovirus genome employing the Tn7 transposition system (Luckow *et al.*, 1993). The  
15 polypeptide product is then expressed to form a functional juvenile hormone-binding polypeptide and preferably, a modulator of a juvenile hormone receptor. The correctly-folded truncated USP polypeptide is used for X-ray and NMR structural analysis and for high-throughput screens.

20

### EXAMPLE 16

#### ***In-vitro* Screening for the Detection of Insecticidal Compounds**

The EcR partner protein (USP polypeptide) of the insect ecdysone receptor and USP polypeptide of the insect juvenile hormone receptor of the present invention, optionally  
25 associated with the EcR polypeptides of insect ecdysone receptors of the present invention as described in the preceding Examples, are coupled to pins according to the procedure of Geysen *et al.* (1987), and reacted with candidate insecticidal compounds, generally at a concentration in the range from about 0.05  $\mu$ M to about 100  $\mu$ M of the candidate compound. The binding of compounds is detected using standard procedures, and compounds having  
30 insecticidal activity are identified. Preferably, such compounds exhibit insecticidal activity against a range of insects, including diptera, hemiptera, coleoptera, ants, and moths, amongst

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others. More preferably, the compounds will exhibit insecticidal activity against *L. cuprina*, *M. persicae*, *D. melanogaster*, scale insect, white fly, and leaf hopper, amongst others. In a particularly preferred embodiment, insecticidal compounds are specific to *L. cuprina* or *M. persicae* and close relatives thereof.

5

### EXAMPLE 17

#### Cloned *Myzus persicae* EcR/USP complex binds ponasterone A *in vitro*.

*In vitro*-translated *Myzus persicae* EcR (MpEcR) polypeptide and an *in vitro*-translated *M. persicae* USP (MpUSP) polypeptide were produced labelled with [<sup>35</sup>S]Methionine, using the  
10 Promega TNT-Coupled Reticulocyte Lysate System. Each batch of lysate contained 100-200 mg/ml of endogenous proteins (using BSA as a standard). The products were analysed by SDS-PAGE and radioautography. The results confirmed that the cloned cDNAs encode proteins of the sizes predicted from the length of putative open reading frames of the cDNAs present in plasmids pMpEcR and pMpUSP. The yields of EcR and USP were similar as  
15 assessed by SDS-PAGE.

In functional assays, DNA plasmids pMpEcR (AGAL Accession No. NM99/04567; 1 mg) or pMpUSP (AGAL Accession No. NM99/04568; 1 mg) or pMpUSP2 (AGAL Accession No. NM00/12581; 1 mg), which have been constructed using the vector pBK-CMV, and 1 ml of  
20 appropriate TNT RNA Polymerase were added to 48 ml of reaction mix which contained TNT Lysate, TNT Reaction Buffer, amino acid mixture, Rnasin Ribonuclease Inhibitor and nuclease-free water in volumes specified in the manufacturer's protocol. In control reactions, a Luciferase T3 control DNA (Promega) was used in place of pMpEcR or pMpUSP. T7 RNA Polymerase was used for transcription of the *M. persicae* EcR RNA from plasmid pMpEcR,  
25 whilst T3 RNA Polymerase was used for transcription of *M. persicae* USP RNA from the plasmid pMpUSP and the Luciferase T3 control DNA. The reactions were carried out for 90 minutes at 30°C.

The control reaction produces 150-500 ng of luciferase per 50 ml reaction.

30

The ecdysteroid binding activities of an *in vitro*-translated *Myzus persicae* EcR (MpEcR)

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polypeptide and an *in vitro*-translated complex of the *M. persicae* EcR and USP polypeptides were produced from the RNAs using the TNT-Coupled Reticulocyte Lysate System (Promega). The mixtures were stored at -20°C overnight.

- 5 After thawing the translation products, 15 ml aliquots of the reaction mixture containing *M. persicae* EcR and USP polypeptides were combined to promote formation of the EcR/USP complex. For assays of individual proteins, 15 ml of the reaction mixture containing *M. persicae* EcR polypeptide or 15 ml of the reaction mixture containing *M. persicae* USP polypeptide was combined with 15 ml of control luciferase protein reaction mixture. Samples were each diluted  
10 to 435 ml with EcR40 buffer [40 mM KCl, 25 mM HEPES pH 7.0, 1 mM EDTA, 1mM DTT, BSA (0.5 mg/ml), 10% glycerol] to allow for triplicates in the ligand binding assay. A control reaction (Blank) was established which contained EcR40 buffer only. An aliquot (140 ml) of each diluted sample was incubated with tritiated ponasterone A (DuPont NEN, Batch Number 3281108) at a final concentration of 2.2 nM for 90 min at room temperature. After incubation, the ligand  
15 binding reactions were placed on ice. The samples were pipetted onto Whatman GF/C filters and incubated for 30 sec. The filters were then placed on a vacuum sinter, washed with 10 ml EcR40 buffer and transferred to scintillation vials. After adding 7 ml of InstaGel Plus to each vial, the contents were vortexed and left at room temperature until the filters became transparent. The receptor bound ligand was quantified using a TriCarb 2100TR scintillation  
20 counter.

The results depicted in Figure 4 indicate that significantly higher amounts of ponasterone A bind to the complex than to either the USP or EcR polypeptides alone.

25

#### EXAMPLE 18

***In vivo* function of a chimeric *L. cuprina* ecdysone receptor and a *L. cuprina* EcR partner protein (USP polypeptide)**

##### Construction of plasmid pSGLcUSP

- 30 A 2453 bp fragment from the 5' end of clone pLSP4 (Example 11), containing nucleotide sequence encoding the *L. cuprina* EcR partner protein (USP polypeptide), was subcloned into

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the *EcoRI* site of the mammalian expression vector pSG5 (Stratagene), to construct pSGLcUSP.

#### Construction of plasmid pVPLcEcR

5 Plasmid pVPLcEcR was constructed as follows:

To construct plasmid pMOD31, plasmid pNLVP16 (a gift from Dr G. Muscat) was digested with *Sall* and *XbaI*, and re-ligated using a double-stranded oligonucleotide linker formed by annealing of the following complementary oligonucleotides:

10

SPX5: 5'-TCGACATATAACTTCGCTGCAGATGCATCCGAGCTCT-3' (SEQ ID NO: 29); and  
XPS3: 5'-CTAGAGCTCGGATGCATCTGCAGCGAAGTTATATG-3' (SEQ ID NO: 30),

The A/B domain of pSGLcEcR was removed from the EcR-encoding cDNA by digestion using  
15 the restriction enzymes *BamHI* and *PstI*, and a 263 bp *BglII/PstI* fragment of plasmid pMOD31, containing a VP16 activation domain (Triezenberg *et al.*, 1988), was ligated in its place, to construct plasmid pVPLcEcR. Accordingly, plasmid pVPLcEcR contains nucleotide sequences encoding the ligand binding region of the *L. cuprina* EcR polypeptide placed operably in connection with the VP16 activation domain.

20

#### Transfection of CV1 Cells

CV1 cells were cotransfected with (i) plasmid pSGLcUSP or unmodified plasmid pSG5, at 1 µg/ml; (ii) plasmid pVPLcEcR or unmodified plasmid pSG5, at 0.2 µg/ml; (iii) the CAT reporter plasmid p(EcRE)<sub>7</sub>-CAT (Example 9), at 1 µg/ml; and (iv) an independent LacZ reporter  
25 plasmid, pPGKLacZ (Example 9), at a concentration of 1ug/ml, included as a control to monitor transfection efficiency.

30

For induction experiments, the ecdysone analogue, 1mM ponasterone A was added to cells 6 hours after transfection. In control experiments, cells were treated only with carrier ethanol.

The CAT and β-galactosidase activities present in extracts of cells were measured 48 hours

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after transfection as described previously (Hannan and Hill, 1997). Variations between experiments were controlled, by normalising the level of CAT to  $\beta$ -galactosidase for each extract.

- 5 Data shown in Figure 5 indicate that the *L. cuprina* EcR partner protein (USP polypeptide) can interact with a chimeric *L. cuprina* EcR polypeptide to form an ecdysteroid-dependent transcription factor in mammalian cells. Treatment of CV1 cells with ecdysteroid, in particular 1mM ponasterone A, induced significant levels of expression of the CAT reporter gene relative to the induction of  $\beta$ -galactosidase gene expression, which indicates transfection efficiency.
- 10 When both plasmid pSGLcUSP and plasmid pVPLcEcR were transfected into CV1 cells, a 73-fold induction of CAT reporter gene expression, relative to  $\beta$ -galactosidase gene expression was achieved (column 8 of Figure 5).

- In contrast, plasmid pVPLcEcR alone produced only a 4-fold induction of CAT gene expression
- 15 relative to  $\beta$ -galactosidase gene expression (column 4 of Figure 5). This low level of activity is presumably due to formation of an active complex by the chimeric *L. cuprina* EcR polypeptide and endogenous RXR present in CV1 cells.

- Only background CAT reporter gene expression was observed in the absence of exogenous
- 20 hormone (columns 1, 3, 5 and 7 of Figure 5), and no significant induction of gene expression was observed in the absence of the *L. cuprina* EcR polypeptide (columns 1, 2, 5 and 6 of Figure 5).

- Overall, these data support the conclusion that the cDNA clone described herein encodes an
- 25 intact *L. cuprina* EcR partner protein (USP polypeptide), which is functional *in vivo*.

#### EXAMPLE 19

##### ***In vivo* function of a chimeric EcR polypeptide of the *M. persicae* ecdysone receptor**

- Plasmids pSGDM and pSGMD both comprise nucleotide sequences encoding chimeric *D. melanogaster* and *M. persicae* ecdysone receptor EcR polypeptides. In particular, plasmid
- 30 pSGDM comprises a chimeric cDNA sequence consisting of nucleotide sequence encoding the



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A/B domain of the *D. melanogaster* EcR polypeptide ligated to nucleotide sequence encoding the DNA-binding, linker, and ligand-binding domains of the *M. persicae* EcR polypeptide. Plasmid pSGMD comprises a chimeric cDNA sequence consisting of nucleotide sequence encoding the A/B domain of the *M. persicae* EcR polypeptide ligated to nucleotide sequence  
5 encoding the DNA-binding, linker, and ligand-binding domains of the *D. melanogaster* EcR polypeptide.

#### Construction of plasmid pSGDM

cDNA encoding the *M. persicae* EcR polypeptide in the mammalian expression vector pSG5  
10 (Stratagene) was digested with *SapI*, which cleaves at a unique restriction site very close to the 3' -end of the nucleotide sequence encoding the A/B domain of the protein (Vector1). Two oligonucleotides A and B, containing *SacII*, *EcoRV* and *BamHI* restriction sites, were synthesized, purified and annealed to form double stranded DNA (Linker1) having *SapI* compatible sticky ends:

- 15 A: 5'-TCCAGAACCGCGGATAGATATCTGGGATCCTC-3' (SEQ ID NO: 31); and  
B: 5'-GGAGAGGATCCCAGATATCTATCCGCGGTTCT-3' (SEQ ID NO: 32)

Linker1 was ligated into Vector 1 and the resultant plasmid was digested with *EcoRV* to produce Vector 2.

20

A 940 bp *EcoRI* cDNA fragment encoding the A/B domain of full length *D. melanogaster* EcR polypeptide was isolated from plasmid pSGDmEcR and ligated into the *EcoRV* site in Vector 2, using the linker-primer from the Stratagene cDNA Synthesis Kit, to produce Vector 3. The cDNA sequence encoding the A/B domain of the *M. persicae* EcR polypeptide was removed  
25 from Vector 3 by digestion with *SacII* and the truncated plasmid was then religated to produce plasmid pSGDM.

#### Construction of plasmid pSGMD.

A 2200 bp *EcoRI/BamHI* cDNA fragment encoding the DNA-binding and ligand-binding  
30 domains of the full length *D. melanogaster* EcR polypeptide was isolated from plasmid pSGDmEcR and end-filled and ligated into the *EcoRV* site present in Vector 2 (see above), to

- 30 A vector for the baculovirus-directed co-expression of ligand-binding regions derived from the  
EcR protein and partner protein (USP polypeptide) of the *Myzus persicae* ecdysone receptor

Linker 2 was constructed by annealing the following oligonucleotides:

Oligonucleotide C:

5'-CCGGGATCTCGAGATGGACTACAAGGACGACGATGACAAGCC-3' (SEQ ID NO: 35); and

Oligonucleotide D:

5'-CATGGGCTTGTCATCGTCGTCCTTGTAGTCCATCTCGAGATC-3' (SEQ ID NO: 36).

- 5 Linker 2 comprises *Xma*I and *Nco*I compatible ends and a "FLAG" encoding sequence.

Linker 2 was ligated to a 1.2kb *Kpn*II/*Nco*I DNA fragment in Vector 1B, encoding the linker and ligand binding domains of the *M. persicae* partner protein (USP polypeptide), to produce plasmid pMpEcR.USP.DUAL, comprising nucleotide sequences encoding the tagged linker and  
10 domains D and E and F of the *M. persicae* EcR polypeptide, and the linker and ligand-binding domains of the *M. persicae* partner protein (USP polypeptide), placed operably under the control of polyhedrin and p10 promoters, respectively.

Plasmids pMpEcR.LcUSP.DUAL and pMpEcR.USP.DUAL were sequenced to confirm the  
15 presence of the open reading frames.

The segment of pMpEcR.LcUSP.DUAL or pMpEcR.USP.DUAL encoding the chimeric tagged ligand binding regions of the receptor polypeptides was recombined in a baculovirus genome, by employing the Tn7 transposition system (Luckow *et al.*, 1993). The chimeric ligand binding  
20 regions of the recombinant ecdysone receptors were then expressed in insect Sf9 cells, where they associated into functional complexes.

Expression of the heterologous *M. persicae*/*L. cuprina* ecdysone receptor [i.e. comprising tagged linker and domains D/E/F of the *M. persicae* EcR polypeptide and the linker and ligand-  
25 binding domains of the *L. cuprina* EcR partner protein (USP polypeptide)], and expression of the homologous *M. persicae* ecdysone receptor [i.e. comprising tagged Linker 2 and domains D/E/F of the *M. persicae* EcR polypeptide and the linker and ligand-binding domains of the *M. persicae* EcR partner protein (USP polypeptide)], was examined by immunoblot analysis of extracts derived from insect Sf9 cells infected with either of the recombinant baculoviruses,  
30 employing antibodies directed against the oligo-His and FLAG tags to perform the quantitation. This analysis detected bands on immunoblot analysis of the predicted sizes for the expressed

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polypeptides.

Furthermore, binding assays, carried out by a modification of the method of Yund *et al.* (1978), demonstrated a highly-significant binding of the tritiated ecdysone analogue, ponasterone A, in cells infected by recombinant viruses (Figure 7). Data presented in Figure 7 indicate correct folding and association of the components of both the heterologous and homologous truncated ecdysone receptors. These data indicate further that it is possible to produce functional heterologous chimeric receptors between the ligand binding regions of EcR polypeptides and EcR partner proteins from different insect species. Those chimeric receptors have different specificities for ecdysteroid compared to their native counterparts.

#### EXAMPLE 21

##### ***In vivo* function of a heterodimeric receptor comprising a chimeric *M. persicae* EcR polypeptide and a recombinant *M. persicae* EcR partner protein (USP polypeptide**

To test the function of the isolated cDNA clone encoding the *M. persicae* EcR partner protein (USP polypeptide), we tested the ability of the expressed polypeptide to complement a chimeric *M. persicae* EcR polypeptide in CV1 cells.

Briefly, CV1 cells were co transfected with the following plasmid constructs:

- (i) plasmid pBKMpUSP1, containing the cDNA clone encoding the *M. persicae* EcR partner protein (USP polypeptide) operably in connection with the CMV promoter in pBK-CMV, at 2 µg/ml; or alternatively, a negative control gene construct, plasmid pBSK+, at 2 µg/ml; and
- (ii) plasmid pSGDM, comprising a chimeric cDNA sequence consisting of nucleotide sequence encoding the α/β domain of the *D. melanogaster* EcR polypeptide ligated to nucleotide sequence encoding the DNA-binding, linker, and ligand-binding domains of the *M. persicae* EcR polypeptide (Example 19), at 1 µg/ml; and
- (iii) the CAT reporter gene construct, plasmid p(EcRE)<sub>7</sub>-CAT, comprising the CAT reporter gene placed operably under the control of a promoter sequence containing multiple copies of the *D. melanogaster* hsp27 ecdysone response elements present in plasmid at 1 µg/ml; and

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- (iv) the  $\beta$ -galactosidase reporter gene construct to control for transfection efficiency, designated plasmid pPopNLacZ, and described by Hannan *et al.* (1993), at 0.5  $\mu$ g/ml.

For expression, the ecdysone analogue, 10 mM ponasterone a (a gift from Dr Denis Horn), was added to cells 6 hours after transfection, to induce CAT gene expression mediated the chimeric recombinant ecdysone receptor. In control experiments, cells were treated with ethanol in place of ponasterone a. CAT and  $\beta$ -galactosidase enzyme activities were measured in cell extracts 48 hours after transfection, as described previously (Hannan and Hill, 1997). The relative level of CAT/  $\beta$ -galactosidase for each extract was determined, to normalise the variation in transfection efficiency between samples.

Data presented in Figure 8 indicate that the isolated cDNA encodes a functional *M. persicae* EcR partner protein (USP polypeptide). When pBKMpUSP1 was co transfected with pSGDM into CV1 cells, a 2.6-fold induction of relative CAT gene expression was observed in the presence of 10 mM ponasterone a, relative to the expression observed using plasmid pSGDM in the absence of pBKMpUSP1. The "background" level of gene expression observed for cells expressing plasmid pSGDM in the absence of pBKMpUSP1 is presumably due to formation of an active complex between the chimeric MpEcR polypeptide and the endogenous RXR proteins present in CV1 cells. The induction of CAT expression by ponasterone a for cells transfected with both plasmids pBKMpUSP1 and pSGDM indicates that the expressed *M. persicae* EcR partner protein (USP polypeptide) can interact with the chimeric EcR polypeptide, to form an ecdysteroid-dependent transcription factor in mammalian cells. Accordingly, these data indicate that the recombinantly-expressed *M. persicae* EcR partner protein (USP polypeptide) is functional *in vivo*.

25

## EXAMPLE 22

### Isolation and characterisation of a cDNA encoding the EcR partner protein (USP polypeptide) of the *Bemisia tabaci* ecdysone receptor

#### Construction and screening of *B. tabaci* cDNA libraries

Two independent *B. tabaci* cDNA libraries derived from red-eye nymph stage animals were prepared by oligo-dT priming, and cloned into the *Eco*RI site of the Lambda/ZapII vector

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(Stratagene). The titres of the two primary libraries produced were  $1.9 \times 10^6$  pfu, and  $3.15 \times 10^6$  pfu. Tests indicated that the insert size range for these libraries was 0.7 kb to 7.6 kb in length.

The primary libraries generated were subsequently amplified according to the manufacturer's instructions, using standard protocols, to produce final titres of  $1.5 \times 10^9$  pfu/ml, and  $2.5 \times 10^9$  pfu/ml.

The prepared cDNA libraries were screened by lifting 500,000 plaques from each amplified cDNA library, in duplicate, onto Hybond N membranes (Amersham), and then hybridizing same under low stringency conditions to radiolabelled probes specified below. In particular, hybridisations were carried out overnight at  $37^\circ\text{C}$ , in a hybridisation solution comprising 42% (w/v) formamide; 5 x SSPE solution; 5 x Denhardt's solution; and 0.1% (w/v) sodium dodecyl sulphate, as described essentially by Ausubel *et al.* (1992) or Sambrook *et al.* (1989). The membranes were then washed at  $37^\circ\text{C}$  in 2XSSC solution containing 0.1% (w/v) sodium dodecyl sulphate. Following washing, positive plaques were detected by autoradiography, using XOMAT-AR film (Kodak) for two to three days, at  $-70^\circ\text{C}$ . Positive-hybridising plaques were plaque-purified, rescued as plasmids, and their cDNA inserts analysed by nucleotide sequence determination.

## 20 Hybridisation probe preparation

The EcR partner protein (USP polypeptide) subunit of the *B. tabaci* ecdysone receptor also functions in the absence of the EcR polypeptide as a USP polypeptide of the *B. tabaci* juvenile hormone receptor.

25 To isolate a cDNA encoding both receptor activities from the *B. tabaci* cDNA library, a 140 bp probe was amplified from *B. tabaci* genomic DNA, using two degenerate primers described by Tzertzinis *et al.* (1994) and in the preceding Examples. The PCR reaction was performed using 1 unit *TaqI* polymerase (Boehringer Mannheim), 1 mM each primer, in a 50  $\mu\text{l}$  reaction volume, essentially under conditions recommended by the manufacturer (Boehringer Mannheim ).

30

The amplified DNA fragment was sub-cloned into the *EcoRI* and *ClaI* sites of linearised

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pBluescript SK+ (Stratagene) vector. The nucleotide sequence of the insert in the pBluescript SK+ vector was obtained using automated fluorescent dye terminator sequencing (Automated DNA Analysis Facility at University of NSW, Sydney Australia) and is set forth herein as SEQ ID NO: 37. This fragment encodes the amino acid sequence set forth in SEQ ID NO: 38.

5

To prepare a hybridisation probe for screening cDNA libraries, the amplified *B. tabaci* DNA was released from the pBluescript+ vector by double-digestion using the enzymes *EcoRI* and *Sall*, separation by agarose gel electrophoresis, and purification by electro-elution. DNA was subsequently [<sup>32</sup>P]-labelled using the GIGAprime DNA Labelling Kit (Bresatec Limited, Adelaide, Australia) essentially according to the manufacturer's instructions, except that random primers were replaced with the degenerate primers described by Tzertzinis *et al.* (1994). Unincorporated label was removed by size exclusion chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used as described herein above at in Examples 7 and 8, to screen the *B. tabaci* cDNA library.

15

Positive-hybridising clones were plaque-purified and sequenced using standard procedures as described herein.

The nucleotide sequence of one clone was obtained and is set forth herein as SEQ ID NO: 39.

20 The amino acid sequence of the *B. tabaci* EcR partner protein (USP polypeptide) is shown in SEQ ID NO: 40.

### EXAMPLE 23

#### Cloning and characterization of a cDNA molecule encoding the EcR polypeptide of the *B. tabaci* ecdysone receptor

25

##### Hybridisation probe preparation

A 101 bp DNA fragment, encoding the DNA-binding domain of the EcR polypeptide subunit of the *B. tabaci* ecdysone receptor, was amplified from the *B. tabaci* genome by PCR, by using the degenerate primers Rdna3 (SEQ ID NO: 23) and Rdna4 (SEQ ID NO: 24), essentially as described hereinabove. Briefly, amplification reactions employed *TaqI* DNA polymerase (Boehringer Mannheim) and the following amplification conditions:

30





## REFERENCES

1. Amann and Brosius (1985). *Gene* 40: 183.
2. Ausubel, et al (1992), *Current Protocols in Molecular Biology*, Greene/Wiley, New York
3. Bugg, et al (1993) *Scientific American*, December Issue: pp 60-66.
- 5 4. Cavener et al. (1987) *Nucl. Acids Res.* 15:1353-136.
5. Devereux, J., et al. (1984). *Nucl. Acids Res.* 12:387-395.
6. Evans (1988) *Science*, 240: 889-895.
7. Geysen et al (1987) *J. Immunol Methods*, 102, 259-274.
8. Greene et al. (1988) *Nucleic Acids Res.* 15:369.
- 10 9. Hannan et al (1993) *Gene* 130 : 233-239
10. Hollenberd and Evans (1988) *Cell* 55: 899-906.
11. Hollenberg et al (1991) *Cell*, 67, 59-77.
12. Jones, G and Sharp, P, (1997) *Proc. Nat. Acad. Sci. USA* 94:13499-13503.
13. Kamimura et al (1996) *Comp. Biochem. Physiol.* 113, 341-347.
- 15 14. Koelle et al (1991) *Cell*, 67:59-77
15. Krust et al (1986) *EMBO. J.*, 5: 891-897.
16. Luckow et al, (1993) *J. Virol*, 67: 4566.
17. McBurney et al (1991), *Nucleic Acids Res.*, 19, 5755-5761.
18. McPherson, M.J., Quirke, P. and Taylor, G.R. (1991) *PCR a Practical Approach*. IRL
- 20 Press, Oxford University Press, Oxford, United Kingdom.
19. Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453.
20. Potrykus (1990) *Bio/Technology* 8: 535-542.
21. Riddihough, G., and Pelham, H. R. B., (1987), *EMBO J.*, 6, 3729-3734
22. Sambrook et al (1989), *Molecular Cloning: a Laboratory Manual*, CSH Lab. Press
- 25 23. Segraves and Hogness (1990) *Genes Devel.* 4: 204-219.
24. Shimatake and Rosenberg (1981) *Nature* 292: 128
25. Studier and Moffat (1986) *J. Mol. Biol.* 189: 113
26. Thompson, et al., (1994) *Nucl. Acids Res.* 22:4673-4680.
27. Tzertzinis et al (1994) *J. Mol. Biol.*, 258, 479-486.
- 30 28. Von Itzstein et al (1993) *Nature* Vol. 363: 418-423.
29. Yund, et al. (1978) *Proc. Nat. Acad. Sci. USA*, 24: 6039-6043.

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**WE CLAIM:**

1. An isolated nucleic acid molecule encoding a member selected from the group consisting of:
  - (i) *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide set forth in SEQ ID NO: 4 or SEQ ID NO: 6 or SEQ ID NO: 8;
  - (ii) *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence encoded by the DNA of *L. cuprina* contained in the plasmid deposited under AGAL Accession No. NM99/04565;
  - (iii) *M. persicae* ecdysteroid receptor EcR polypeptide set forth in SEQ ID NO: 14;
  - (iv) *M. persicae* ecdysteroid receptor EcR polypeptide sequence encoded by the DNA of *M. persicae* contained in the plasmid deposited under AGAL Accession No. NM99/04567;
  - (v) *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 18 or SEQ ID NO: 20;
  - (vi) *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence encoded by the DNA of *M. persicae* contained in the plasmid deposited under AGAL Accession No. NM99/04568 or AGAL Accession No. NM00/12581;
  - (vii) insect ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide having at least about 90% identity to the *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) fragment set forth in SEQ ID NO: 38 or at least about 80% identity to the *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 40;
  - (viii) insect ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide having at least about 80% identity to the *B.*

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*tabacai* ecdysteroid receptor partner protein (USP polypeptide) sequence encoded by the DNA of *B. tabacai* contained in the plasmid deposited under AGAL Accession No. NM00/12580;

- (ix) *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide encoded by nucleic acid that is capable of hybridizing under at least moderate stringency conditions to the DNA of *B. tabacai* contained in the plasmid deposited under AGAL Accession No. NM00/12580;
  - (x) *B. tabacai* ecdysteroid receptor EcR polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 42;
  - (xi) *B. tabacai* ecdysteroid receptor EcR polypeptide encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO: 41;
  - (xii) an amino acid sequence consisting of the ligand binding region of any one of (i) to (xi), said ligand binding region comprising at least the hormone binding domain and/or a part of the linker domain of any one of (i) to (xi); and
  - (xiii) a fusion polypeptide between the ligand binding region of an ecdysteroid EcR polypeptide and the ligand binding region of an ecdysteroid partner protein (USP polypeptide) wherein at least one of said ligand binding regions comprises at least the hormone binding domain and/or a part of the linker domain of any one of (i) to (xi).
2. The isolated nucleic acid molecule according to claim 1 wherein the ligand binding region comprises a linker domain of said EcR polypeptide or partner protein (USP polypeptide).
3. The isolated nucleic acid molecule according to claim 1 wherein the ligand binding region comprises a hormone binding domain of said EcR polypeptide or partner protein (USP polypeptide).

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4. The isolated nucleic acid molecule according to claim 1 wherein the ligand binding region comprises at least a part of the linker domain and all of the hormone binding domain of said EcR polypeptide or partner protein (USP polypeptide).
5. The isolated nucleic acid molecule according to any one of claims 1 to 4 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor EcR polypeptide of SEQ ID NO 14.
6. The isolated nucleic acid molecule according to any one of claims 1 to 4 wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor EcR polypeptide, said EcR polypeptide comprising the amino acid sequence of SEQ ID NO 42 or encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO: 41.
7. The isolated nucleic acid molecule according to any one of claims 1 to 4 wherein the ligand binding region is the ligand binding region of the *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) comprising an amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 6 and SEQ ID NO: 8
8. The isolated nucleic acid molecule according to any one of claims 1 to 4 wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) comprising the amino acid sequence set forth in SEQ ID NO: 40.
9. The isolated nucleic acid molecule according to any one of claims 1 to 4 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 16.

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10. The isolated nucleic acid molecule according to any one of claims 1 to 4 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 18.
11. An isolated nucleic acid molecule comprising a sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17; SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, the sequence of the *L. cuprina* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04565, the sequence of the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04567, the sequence of the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04568, the sequence of the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM00/12581, the sequence of the *B. tabacai* DNA contained in the plasmid deposited under AGAL Accession No. NM00/12580, a sequence having at least 80% identity to SEQ ID NO: 37, a sequence having at least 60% identity to SEQ ID NO: 39, a sequence having at least 90% identity to SEQ ID NO: 41, a sequence having at least about 80% identity to the *B. tabacai* DNA contained in the plasmid deposited under AGAL Accession No. NM00/12580, and a sequence of *B. tabacai* that hybridizes under moderate stringency conditions to the *B. tabacai* DNA contained in the plasmid deposited under AGAL Accession No. NM00/12580 or to the complement of SEQ ID NO: 37 or SEQ ID NO: 39 or SEQ ID NO: 41.
12. An isolated nucleic acid molecule encoding *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide or the ligand binding region of said polypeptide, said nucleic acid comprising a nucleotide sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 5 or SEQ ID NO: 7;
  - and

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- (ii) the sequence of the *L. cuprina* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04565.
- 13. An isolated nucleic acid molecule encoding *M. persicae* ecdysteroid receptor EcR polypeptide or the ligand binding region of said polypeptide, said nucleic acid comprising a nucleotide sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 13; and
  - (ii) the sequence of the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04567.
- 14. An isolated nucleic acid molecule encoding *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide or the ligand binding region of said polypeptide, said nucleic acid comprising a nucleotide sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 17 or SEQ ID NO: 19; and
  - (ii) the sequence of the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04568 or AGAL Accession No. NM00/12581.
- 15. An isolated nucleic acid molecule encoding *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor or the ligand binding region of said polypeptide, said nucleic acid comprising a nucleotide sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 37 or SEQ ID NO: 39; and
  - (ii) the sequence of the *B. tabacai* DNA contained in the plasmid deposited under AGAL Accession No. NM00/12580.

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16. An isolated nucleic acid molecule encoding *B. tabacae* ecdysteroid receptor EcR polypeptide or the ligand binding region of said polypeptide, said nucleic acid comprising the sequence set forth in SEQ ID NO: 41.
17. A method of identifying nucleic acid encoding an insect ecdysteroid receptor polypeptide comprising:
  - (i) hybridizing genomic DNA, mRNA or cDNA of an insect with a hybridization-effective amount of one or more hybridization probes selected from the group consisting of:
    - (a) a probe comprising at least 10 contiguous nucleotides in length from a nucleotide sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41;
    - (b) a probe comprising at least 10 contiguous nucleotides in length from cDNA contained in the plasmid deposited under AGAL Accession No. NM00/12580;
    - (c) a probe comprising at least 10 contiguous nucleotides from residues 1 to about 450 of SEQ ID NO: 13; and
    - (d) a probe comprising at least 10 contiguous nucleotides of SEQ ID NO: 15 or SEQ ID NO: 17 or SEQ ID NO: 19 that does not include a nucleotides 192 to 323 of SEQ ID NO: 15; and
    - (e) a probe having a sequence complementary to any one of (a) to (d); and
  - (ii) detecting the hybridization.
18. The method of claim 17 wherein the step of detecting the hybridization comprises detecting a reporter molecule that is covalently bound to the probe.
19. A method of identifying nucleic acid encoding an insect ecdysteroid receptor polypeptide comprising:



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- (i) annealing to genomic DNA, mRNA or cDNA one or more PCR primers selected from the group consisting of:
    - (a) a primer comprising at least 10 contiguous nucleotides in length from a nucleotide sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41;
    - (b) a primer comprising at least 10 contiguous nucleotides in length from cDNA contained in the plasmid deposited under AGAL Accession No. NM00/12580;
    - (c) a primer comprising at least 10 contiguous nucleotides from residues 1 to about 450 of SEQ ID NO: 13; and
    - (d) a primer comprising at least 10 contiguous nucleotides of SEQ ID NO: 15 or SEQ ID NO: 17 or SEQ ID NO: 19 that does not include a nucleotides 192 to 323 of SEQ ID NO: 15; and
    - (e) a primer having a sequence complementary to any one of (a) to (d); and
  - (ii) amplifying a nucleotide sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide in a polymerase chain reaction.
20. A method of identifying nucleic acid that encodes an insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide comprising:
- (i) amplifying nucleic acid using one or more PCR primers selected from the group consisting of:
    - (a) a primer comprising at least 10 contiguous nucleotides in length from a nucleotide sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41;
    - (b) a primer comprising at least 10 contiguous nucleotides in length from cDNA contained in the plasmid deposited under AGAL Accession No. NM00/12580;

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- (c) a primer comprising at least 10 contiguous nucleotides from residues 1 to about 450 of SEQ ID NO: 13; and
  - (d) a primer comprising at least 10 contiguous nucleotides of SEQ ID NO: 15 or SEQ ID NO: 17 or SEQ ID NO: 19 that does not include a nucleotides 192 to 323 of SEQ ID NO: 15;
  - (ii) hybridizing the amplified nucleic acid to genomic DNA, mRNA or cDNA of an insect; and
  - (iii) detecting the hybridization.
21. The method of claim 20 wherein detecting the hybridization comprises detecting a reporter molecule that is covalently bound to the amplified nucleic acid.
22. The method according to any one of claims 17 to 21 further comprising isolating the identified nucleic acid molecule.
23. A gene construct comprising the isolated nucleic acid molecule according to any one of claims 1 to 16 operably linked to a promoter sequence.
24. The gene construct according to claim 23 wherein the promoter is selected from the group consisting of SV40 promoter, MMTV promoter, polyhedron promoter and p10 promoter.
25. A recombinant or isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (i) *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 6 or SEQ ID NO: 8;
  - (ii) *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence encoded by the DNA of *L.*

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*cuprina* contained in the plasmid deposited under AGAL Accession No. NM99/04565;

- (iii) *M. persicae* ecdysteroid receptor EcR polypeptide sequence set forth in SEQ ID NO: 14;
- (iv) *M. persicae* ecdysteroid receptor EcR polypeptide sequence encoded by the DNA of *M. persicae* contained in the plasmid deposited under AGAL Accession No. NM99/04567;
- (v) *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 18 or SEQ ID NO: 20;
- (vi) sequence of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence encoded by the DNA of *M. persicae* contained in the plasmid deposited under AGAL Accession No. NM99/04568 or AGAL Accession No. NM00/12581;
- (vii) insect ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide sequence having at least about 90% identity to the *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 38 or at least about 80% identity to the *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 40;
- (viii) insect ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide having at least about 80% identity to the *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) sequence encoded by the DNA of *B. tabacai* contained in the plasmid deposited under AGAL Accession No. NM00/12580;
- (ix) *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide encoded by nucleic acid that is capable of hybridizing under at least moderate stringency conditions to the DNA of *B. tabacai* contained in the plasmid deposited under AGAL Accession No. NM00/12580;

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- (x) *B. tabacai* ecdysteroid receptor EcR polypeptide comprising the *B. tabacai* sequence set forth in SEQ ID NO: 42;
  - (xi) *B. tabacai* ecdysteroid receptor EcR polypeptide encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO: 41;
  - (xii) the ligand binding region of any one of (i) to (xi), said ligand binding region comprising at least the hormone binding domain and/or a part of the linker domain of any one of (i) to (xi); and
  - (xiii) a fusion polypeptide between the ligand binding region of an ecdysteroid EcR polypeptide and the ligand binding region of an ecdysteroid partner protein (USP polypeptide) wherein at least one of said ligand binding regions comprises at least the hormone binding domain and/or a part of the linker domain of any one of (i) to (xi).
26. The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region comprises a linker domain of said EcR polypeptide or partner protein (USP polypeptide).
27. The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region comprises a hormone binding domain of said EcR polypeptide or partner protein (USP polypeptide).
28. The isolated or recombinant polypeptide according to claim 25 wherein the ligand binding region comprises at least a part of the linker domain and all of the hormone binding domain of said EcR polypeptide or partner protein (USP polypeptide).
29. The isolated or recombinant polypeptide according to any one of claims 25 to 28 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor EcR polypeptide of SEQ ID NO: 14.

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30. The isolated or recombinant polypeptide according to any one of claims 25 to 28 wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor EcR polypeptide comprising the amino acid sequence of SEQ ID NO 42 or encoded by nucleic acid that hybridizes under at least moderate stringency conditions to the complement of SEQ ID NO: 41.
31. The isolated or recombinant polypeptide according to any one of claims 25 to 28 wherein the ligand binding region is the ligand binding region of the *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) comprising an amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 6, and SEQ ID NO: 8.
32. The isolated or recombinant polypeptide according to any one of claims 25 to 28 wherein the ligand binding region is the ligand binding region of a *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) comprising the amino acid sequence set forth in SEQ ID NO: 40.
33. The isolated or recombinant polypeptide according to any one of claims 25 to 28 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 16.
34. The isolated or recombinant polypeptide according to any one of claims 25 to 28 wherein the ligand binding region is the ligand binding region of the *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) set forth in SEQ ID NO: 18.
35. A recombinant or isolated *L. cuprina* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide or the ligand binding region of said polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 6 or SEQ ID NO: 8;

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- (ii) the sequence of the ligand binding region of (i); and
  - (iii) the sequence encoded by the *L. cuprina* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04565.
  
- 36. A recombinant or isolated *M. persicae* ecdysteroid EcR polypeptide or the ligand binding region of said polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 14;
  - (ii) the sequence of the ligand binding region of (i); and
  - (iii) the sequence encoded by the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04567.
  
- 37. A recombinant or isolated *M. persicae* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide or the ligand binding region of said polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 18 or SEQ ID NO: 20;
  - (ii) the sequence of the ligand binding region of (i); and
  - (iii) the sequence encoded by the *M. persicae* DNA contained in the plasmid deposited under AGAL Accession No. NM99/04568 or AGAL Accession No. NM00/12581.
  
- 38. A recombinant or isolated *B. tabacai* ecdysteroid receptor partner protein (USP polypeptide) or juvenile hormone receptor polypeptide or the ligand binding region of said polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (i) the sequence set forth in SEQ ID NO: 40;
  - (ii) the sequence of the ligand binding region of (i); and

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- (iii) the sequence encoded by the *B. tabacai* DNA contained in the plasmid deposited under AGAL Accession No. NM00/12580.
39. A recombinant or isolated *B. tabacai* ecdysteroid receptor EcR polypeptide or the ligand binding region of said polypeptide comprising an amino acid sequence selected from the group consisting of:
- (i) a sequence comprising the sequence set forth in SEQ ID NO: 42; and
  - (ii) the sequence of the ligand binding region of (i).
40. A cell comprising the isolated nucleic acid molecule according to any one of claims 1 to 16 or the gene construct according to claim 23 or 24.
41. The cell according to claim 40 being a prokaryotic or eukaryotic cell.
42. The cell according to claim 41, wherein the eukaryotic cell is an insect cell or a mammalian cell.
43. The cell according to claim 42 wherein the insect is *Spodoptera frugiperda* or the mammalian cell is a CHO cell.
44. A cell that expresses the isolated or recombinant polypeptide according to any one of claims 25 to 39.
45. The cell according to claim 44, being an insect cell or a mammalian cell.
46. The cell according to claim 45 wherein the insect cell is derived from *Spodoptera frugiperda* or the mammalian cell is a CHO cell.
47. A method of identifying a modulator of steroid receptor-mediated gene expression or juvenile hormone receptor-mediated gene expression comprising:

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- (i) assaying the expression of a reporter gene in the presence of the recombinant or isolated polypeptide according to any one of claims 25 to 39 and a potential modulator;
- (ii) assaying the expression of the reporter gene in the presence of the recombinant or isolated polypeptide according to any one of claims 25 to 39 and without said potential modulator; and
- (iii) comparing expression of the reporter gene at (i) and (ii) ,

wherein expression of said reporter gene is effected by the binding of said polypeptide to a steroid response element (SRE) or a promoter sequence comprising said SRE, and wherein a different level of expression at (iii) indicates that said potential modulator is a modulator of steroid receptor-mediated gene expression.

- 48. The method according to claim 47, wherein the SRE is the hsp27 ecdysone response element or the 13 bp core palindrome thereof.
- 49. The method according to claim 47, wherein the promoter is the SV40 promoter, MMTV promoter, p10 promoter or polyhedron promoter.
- 50. The method according to any one of claims 47 to 49, wherein the reporter gene is the CAT gene or the  $\beta$ -galactosidase gene.
- 51. The method of claim 47 wherein the modulator of steroid receptor-mediated gene expression or juvenile hormone receptor-mediated gene expression is a steroid receptor antagonist or juvenile hormone receptor antagonist.
- 52. The method of claim 47 wherein the modulator of steroid receptor-mediated gene expression or juvenile hormone receptor-mediated gene expression is a steroid receptor agonist or juvenile hormone receptor agonist.



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53. The method of claim 51 or 52, wherein the agonist or antagonist is a synthetic chemical that mimics the structure of a ligand of said receptor, thereby modulating binding of said ligand to said receptor.
54. The method of claim 53, wherein the synthetic chemical is a bisacylhydrazine insecticide, iridoid glycoside or other non-steroidal modulator of an ecdysteroid receptor or juvenile hormone receptor.
55. A method of identifying a potential insecticidal compound comprising:
- (i) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to any one of claims 25 to 39 to a steroid response element (SRE) to which said polypeptide binds, in the presence of a candidate compound;
  - (ii) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to any one of claims 25 to 39 to a steroid response element (SRE) to which said polypeptide binds, in the absence of said candidate compound; and
  - (iii) comparing the binding assayed at (i) and (ii), wherein a difference in the level of binding indicates that the candidate compound possesses potential insecticidal activity.
56. The method according to claim 55, wherein the binding is assayed indirectly by determining the level of expression of a reporter gene which is placed operably under the control of the steroid response element (SRE) to which the isolated or recombinant polypeptide binds or a promoter sequence comprising said SRE.
57. The method according to claim 56, wherein the SRE is the hsp27 ecdysone response element or the 13 bp core palindrome thereof.

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58. The method according to claim 56, wherein the promoter is the SV40 promoter, MMTV promoter, p10 promoter or polyhedron promoter.
59. The method according to any one of claims 56 to 58, wherein the reporter gene is the CAT gene or the  $\beta$ -galactosidase gene.
60. The method according to any one of claims 55 to 59, wherein the potential insecticidal compound is an insect steroid receptor antagonist or insect juvenile hormone receptor antagonist.
61. The method according to any one of claims 55 to 59, wherein the potential insecticidal compound is an insect steroid receptor agonist or insect juvenile hormone receptor agonist.
62. The method of claim 60 or 61, wherein the agonist or antagonist is a synthetic chemical that mimics the structure of a ligand of an insect steroid receptor or a juvenile hormone receptor, thereby modulating binding of said ligand to said receptor.
63. The method of claim 62, wherein the synthetic chemical is a bisacylhydrazine insecticide, iridoid glycoside or other non-steroidal modulator of an insect ecdysteroid receptor or insect juvenile hormone receptor.
64. A method of identifying a candidate insecticidally-active agent comprising:
- (i) expressing the recombinant or isolated polypeptide of claim 25 wherein said polypeptide is an EcR polypeptide or a ligand binding region comprising at least the hormone binding domain and a part of the linker region of said EcR polypeptide, optionally in association with the partner protein (USP polypeptide) of an insect ecdysteroid receptor or the ligand-binding region thereof so as to form a functional hormone-binding complex;

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- (ii) purifying or precipitating the EcR polypeptide or ligand binding region or hormone binding complex;
  - (iii) determining the three-dimensional structure of the ligand binding domain of the polypeptide or complex; and
  - (iv) identifying a compound that binds to or associates with the three-dimensional structure of the ligand binding region, wherein said compound represents a candidate insecticidally-active agent.
65. The method of claim 64, wherein the candidate insecticidally-active agent is a synthetic chemical that mimics the structure of a ligand of an insect steroid receptor or a juvenile hormone receptor, thereby modulating binding of said ligand to said receptor.
66. The method of claim 65, wherein the synthetic chemical is a bisacylhydrazine insecticide, iridoid glycoside or other non-steroidal modulator of an insect ecdysteroid receptor or insect juvenile hormone receptor.
67. A method of identifying a candidate insecticidally-active agent comprising:
- (i) expressing the recombinant or isolated polypeptide of claim 25 wherein said polypeptide is a partner protein (USP polypeptide) or a ligand binding region comprising at least the hormone binding domain and a part of the linker region of said partner protein (USP polypeptide), optionally in association with the EcR polypeptide of an insect ecdysteroid receptor or the ligand-binding region thereof so as to form a functional hormone-binding complex;
  - (ii) purifying or precipitating the partner protein (USP polypeptide) or ligand binding region or hormone binding complex;
  - (iii) determining the three-dimensional structure of the ligand binding domain of the polypeptide or complex; and

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- (iv) identifying a compound that binds to or associates with the three-dimensional structure of the ligand binding region, wherein said compound represents a candidate insecticidally-active agent.
68. The method of claim 67, wherein the candidate insecticidally-active agent is a synthetic chemical that mimics the structure of a ligand of an insect steroid receptor or an insect juvenile hormone receptor, thereby modulating binding of said ligand to said receptor.
69. The method of claim 68, wherein the synthetic chemical is a bisacylhydrazine insecticide, iridoid glycoside or other non-steroidal modulator of an insect ecdysteroid receptor or insect juvenile hormone receptor.
70. A synthetic compound that interacts with the three dimensional structure of the isolated or recombinant polypeptide according to any one of claims 25 to 39 wherein said compound is capable of binding to said polypeptide or protein to agonize or antagonize the binding activity or bioactivity thereof.
71. A method of identifying a synthetic compound having insecticidal activity comprising contacting the recombinant or isolated polypeptide according to any one of claims 25 to 39 with said compound for a time and under conditions sufficient for binding to occur and detecting said binding using a detection means, wherein the occurrence of binding is indicative of potential insecticidal activity of the compound.
72. A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said hormone-binding complex comprises:

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- (i) the ligand-binding region of an ecdysteroid receptor partner protein (USP polypeptide) according to any one of claims 25 to 28 or any one of claims 31 to 35 or claim 37 or 38; and
  - (ii) the EcR polypeptide of an insect ecdysteroid receptor or the ligand binding region thereof.
73. A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said hormone-binding complex comprises:
- (i) the ligand-binding region of an EcR polypeptide according to any one of claims 25 to 30 or claim 36 or 39; and
  - (ii) the ecdysteroid receptor partner protein (USP polypeptide) of an insect ecdysteroid receptor or the ligand binding region thereof.
74. A hormone-binding complex that binds an insect ecdysteroid or binds a synthetic chemical that mimics the structure of said ecdysteroid, wherein said complex comprises:
- (i) the ligand binding region of an EcR polypeptide according to any one of claims 25 to 30 or claim 36 or 39; and
  - (ii) the ligand binding region of an ecdysteroid receptor partner protein (USP polypeptide) according to any one of claims 25 to 28 or any one of claims 31 to 35 or claim 37 or 38.

10030729 . 062002

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

NSW

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
11 January 2001 (11.01.2001)

PCT

(10) International Publication Number  
**WO 01/02436 A1**

- (51) International Patent Classification<sup>7</sup>: C07K 14/72, C12N 15/12, C07H 21/04
- (21) International Application Number: PCT/AU00/00799
- (22) International Filing Date: 30 June 2000 (30.06.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
09/346,470 1 July 1999 (01.07.1999) US
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, ACT 2601 (AU).
- Published:**  
— With international search report.  
— With (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description.
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): HILL, Ronald, Johnston [AU/AU]; Altona Avenue, Forestville, NSW 2087 (AU). HANNAN, Garry, Noel [AU/AU]; 32 Earl Street, Hunters Hill, NSW 2110 (AU).
- (74) Agents: OLIVE, Mark, R. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/02436 A1

(54) Title: NOVEL GENETIC SEQUENCES ENCODING STEROID AND JUVENILE HORMONE RECEPTOR POLYPEPTIDES AND USES THEREFOR

(57) Abstract: The present invention provides isolated nucleic acid molecules encoding polypeptides comprising functional steroid hormone and juvenile hormone receptors, in particular isolated nucleic acid molecules which encode polypeptides comprising the *Lucilia cuprina*, *Myzus persicae*, and *Bemisia tabaci* ecdysone receptors and juvenile hormone receptors. The present invention further provides functional recombinant steroid and juvenile hormone receptors and recombinant polypeptide subunits thereof and derivatives and analogues thereof. The present invention further provides screening systems and methods of identifying insecticidally-active agents which are capable of agonising or antagonising insect receptor function, or alternatively or in addition, which modify the affinity of said receptors for their cellular stimuli (e.g. insect steroids or juvenile hormones) or analogues thereof, or alternatively or in addition, which act as insecticides by virtue of their ability to agonise or antagonise the activity of insect hormones.

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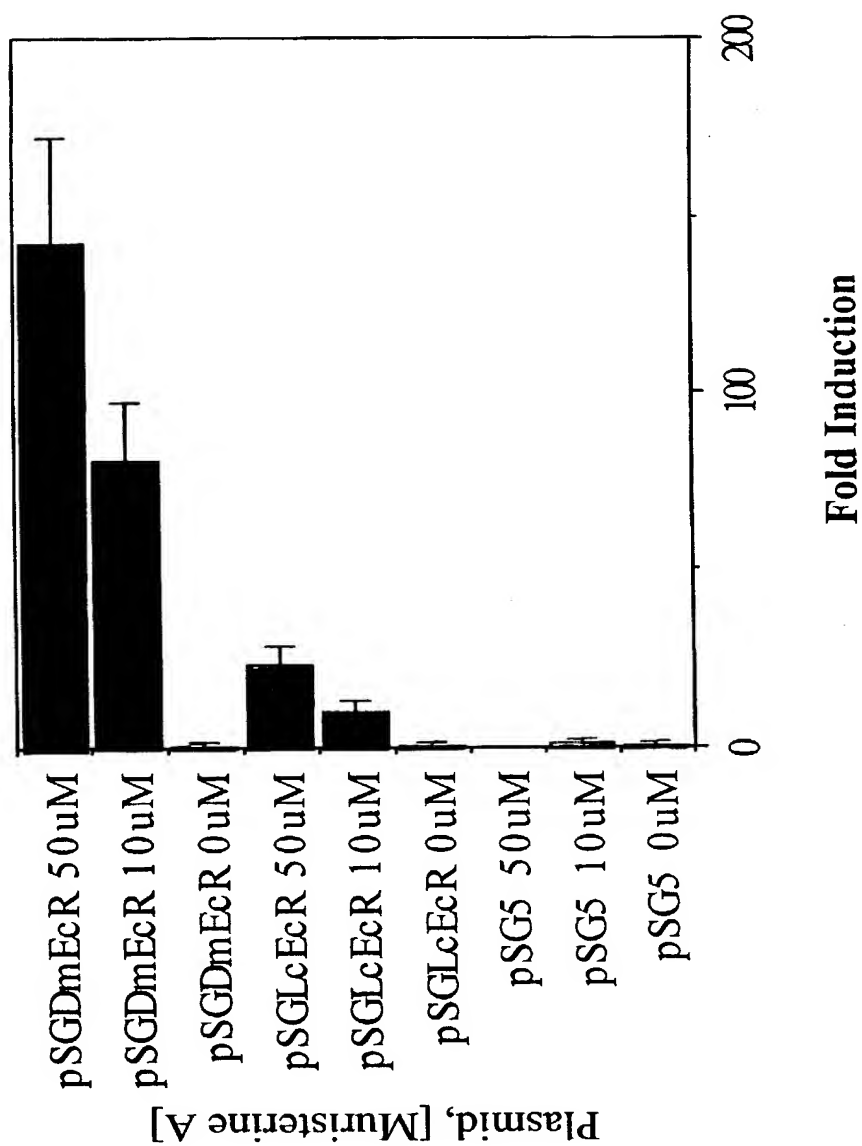


FIGURE 1

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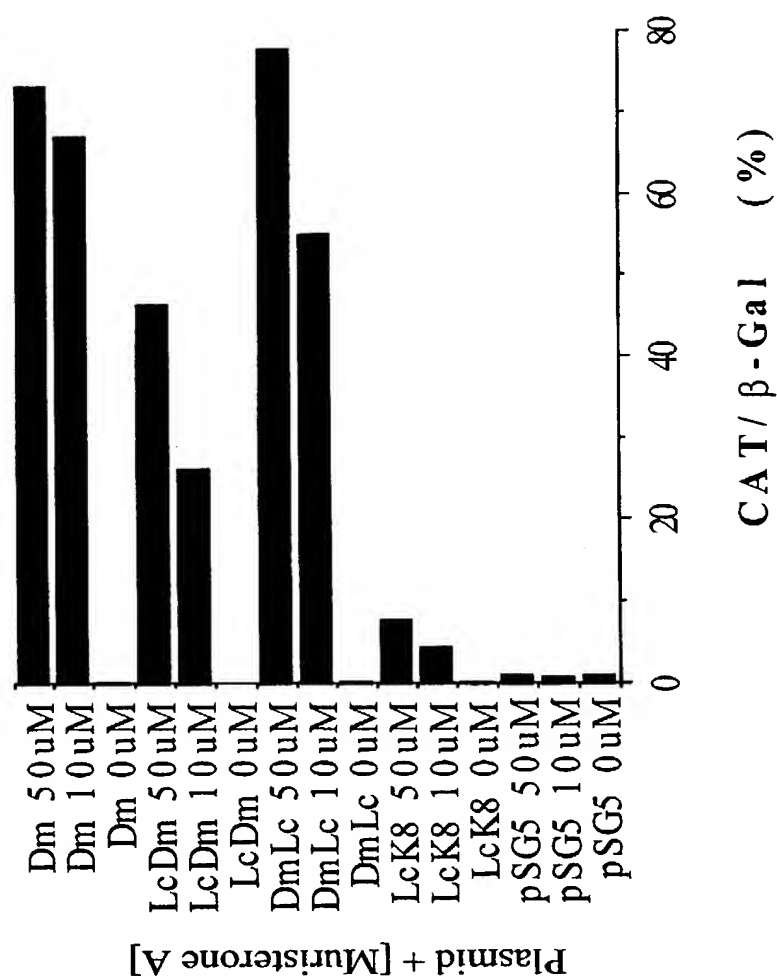


FIGURE 2



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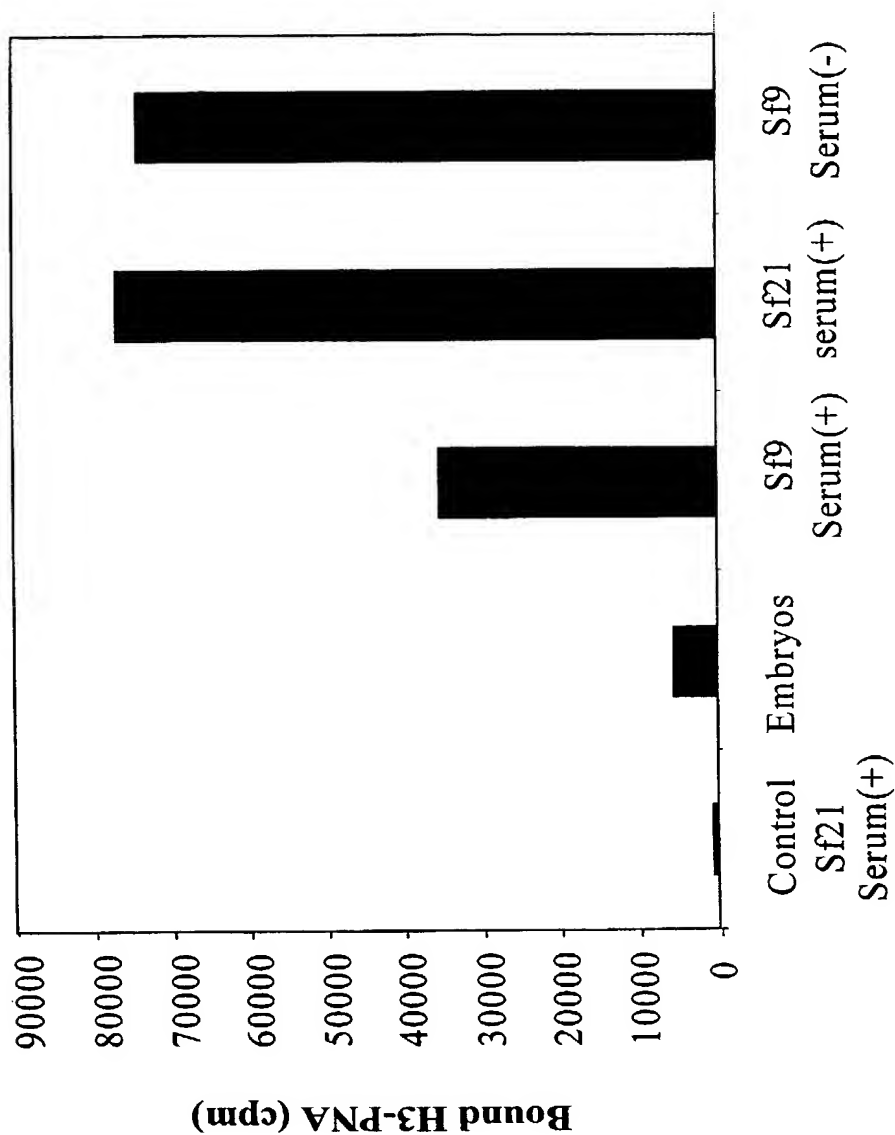


FIGURE 3

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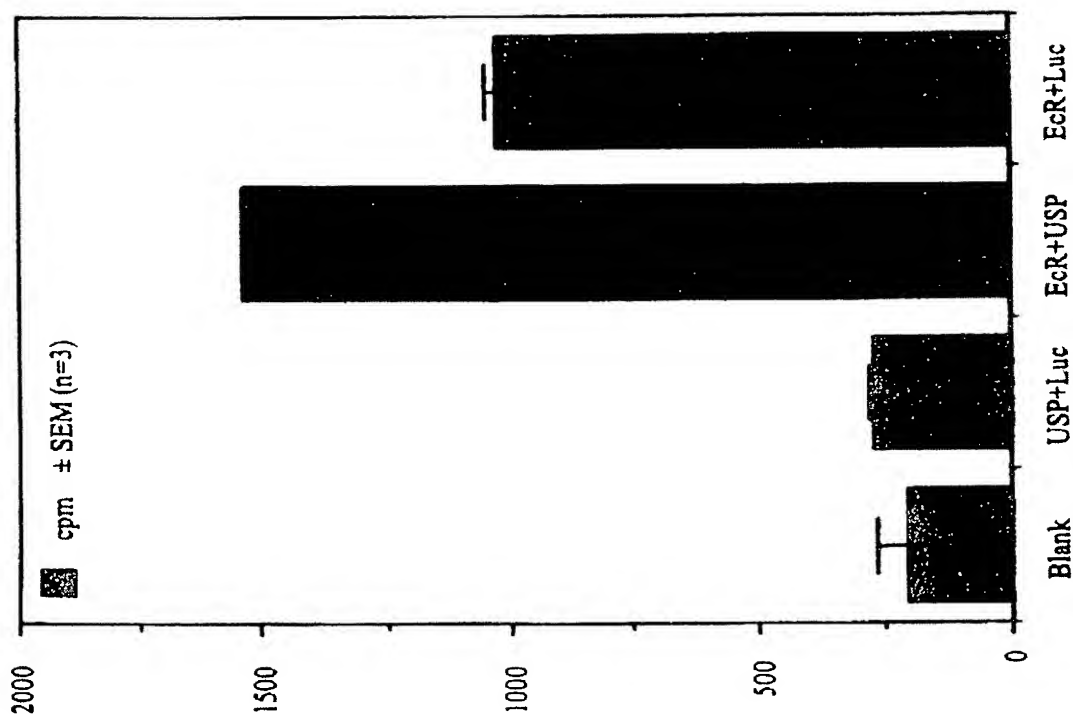


FIGURE 4

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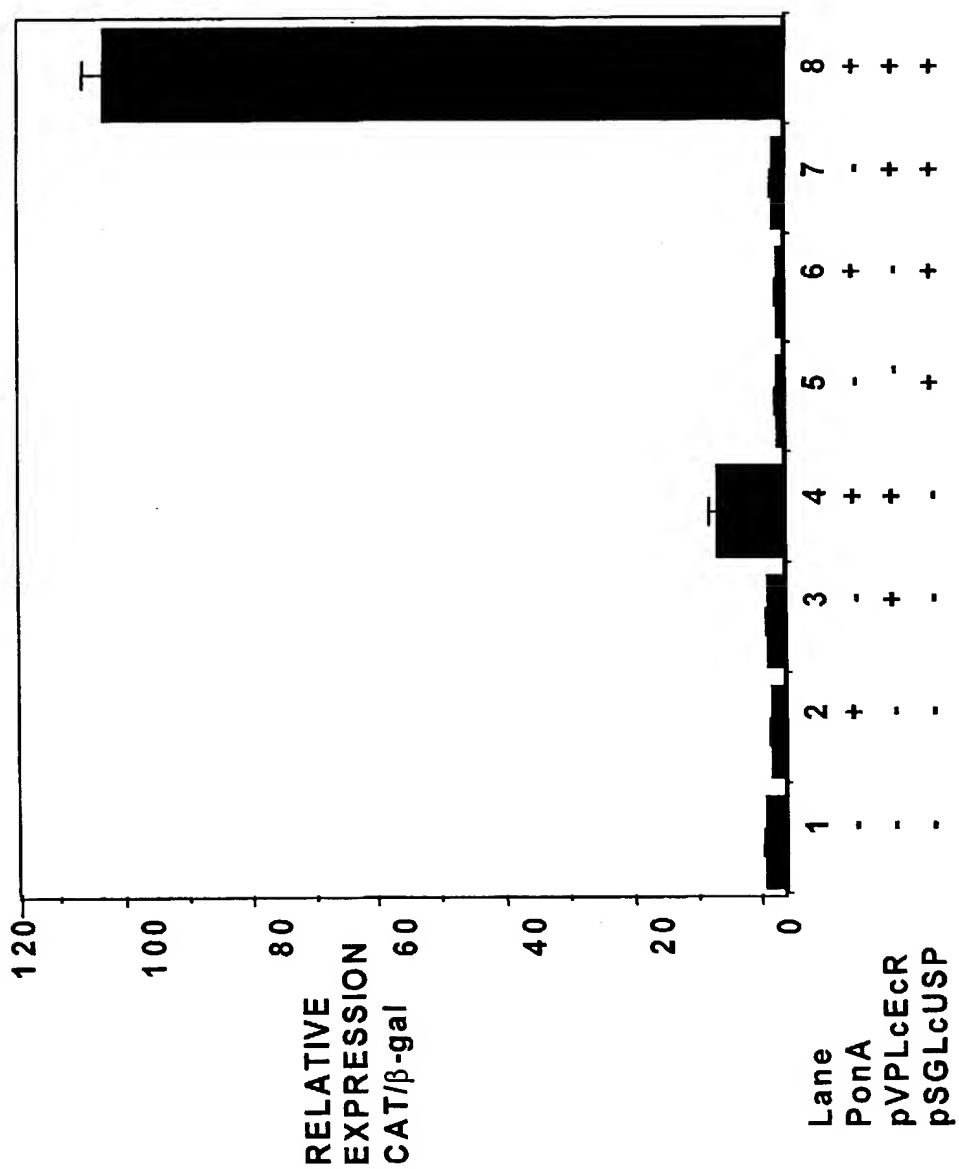


FIGURE 5

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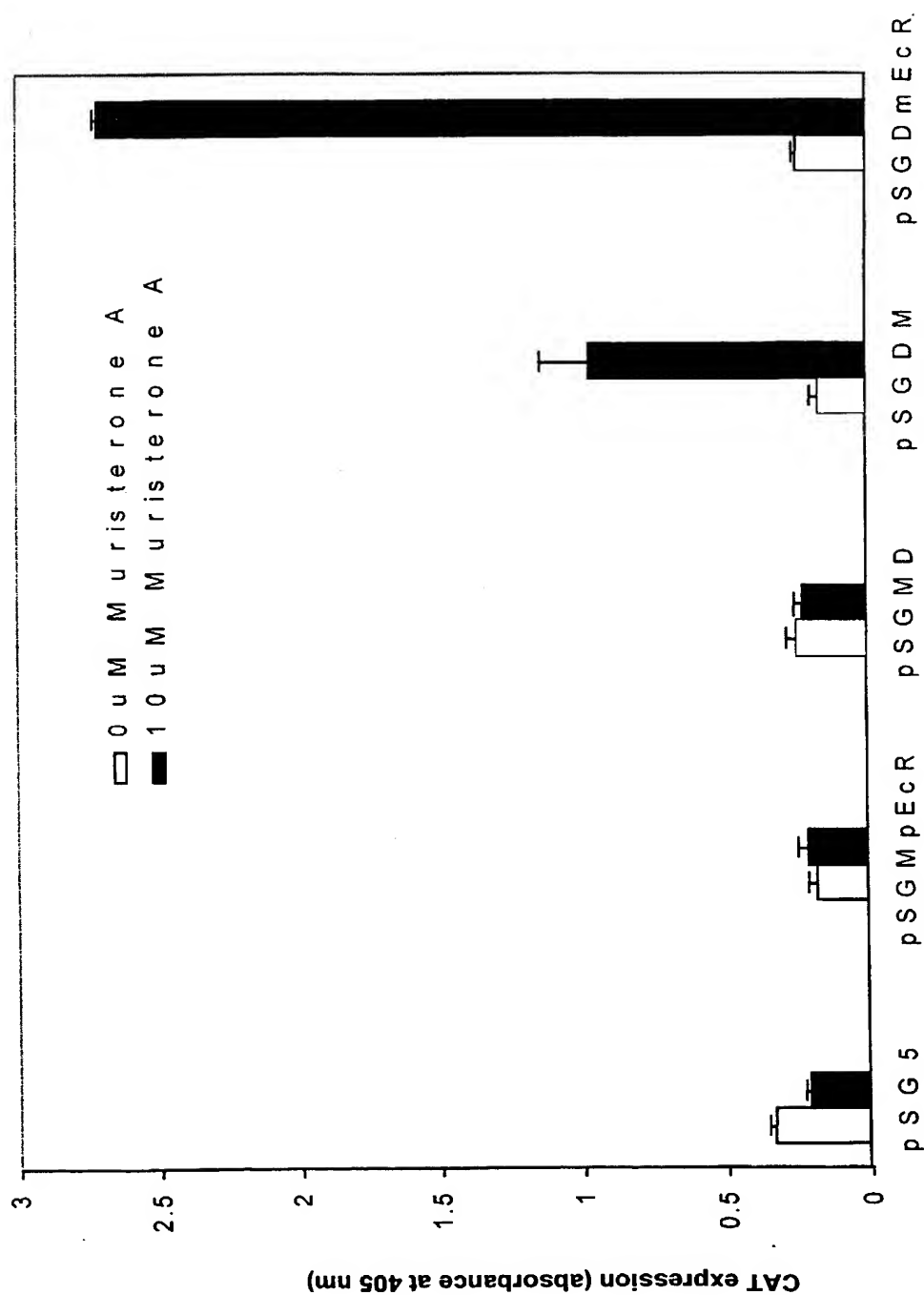
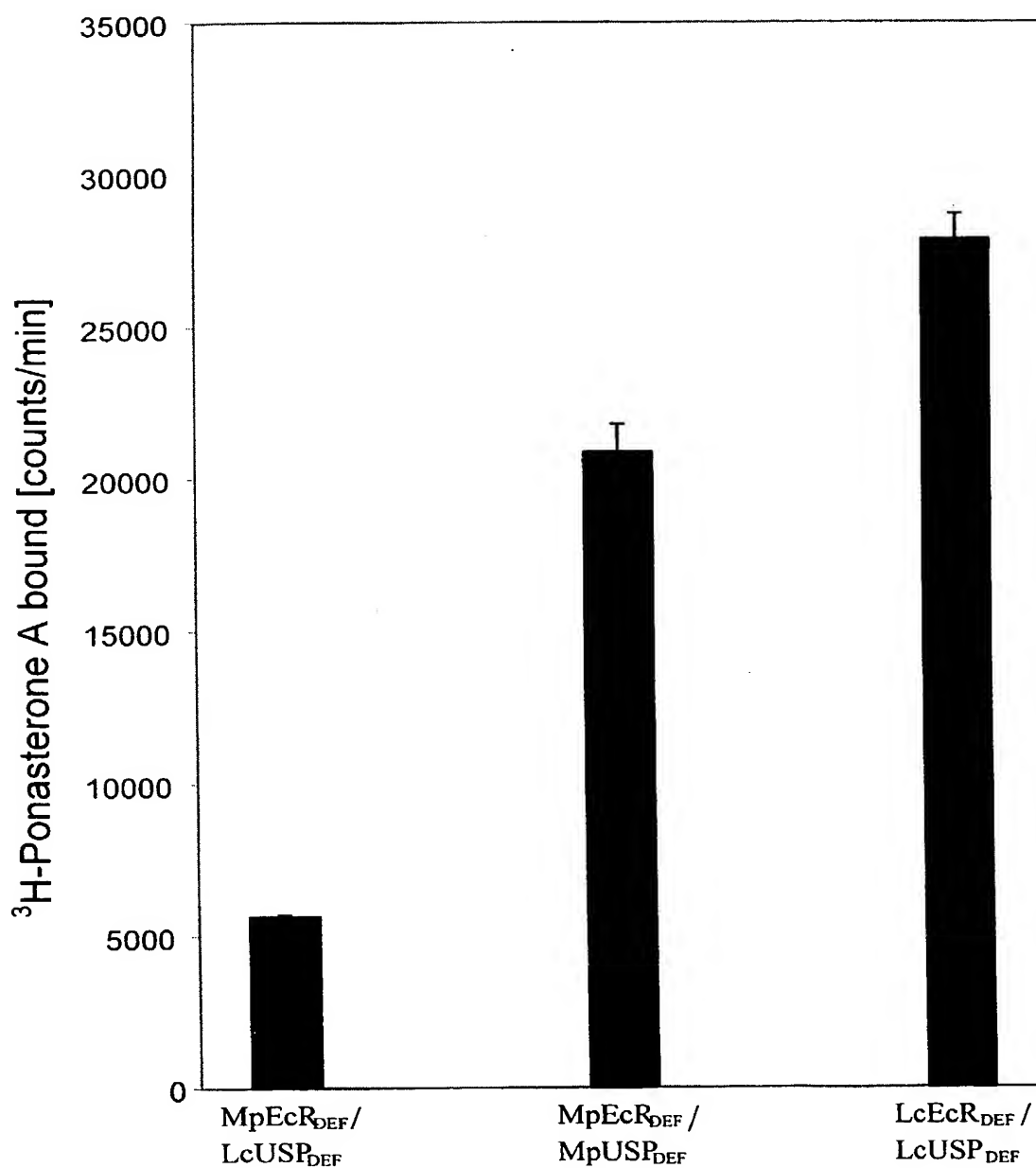
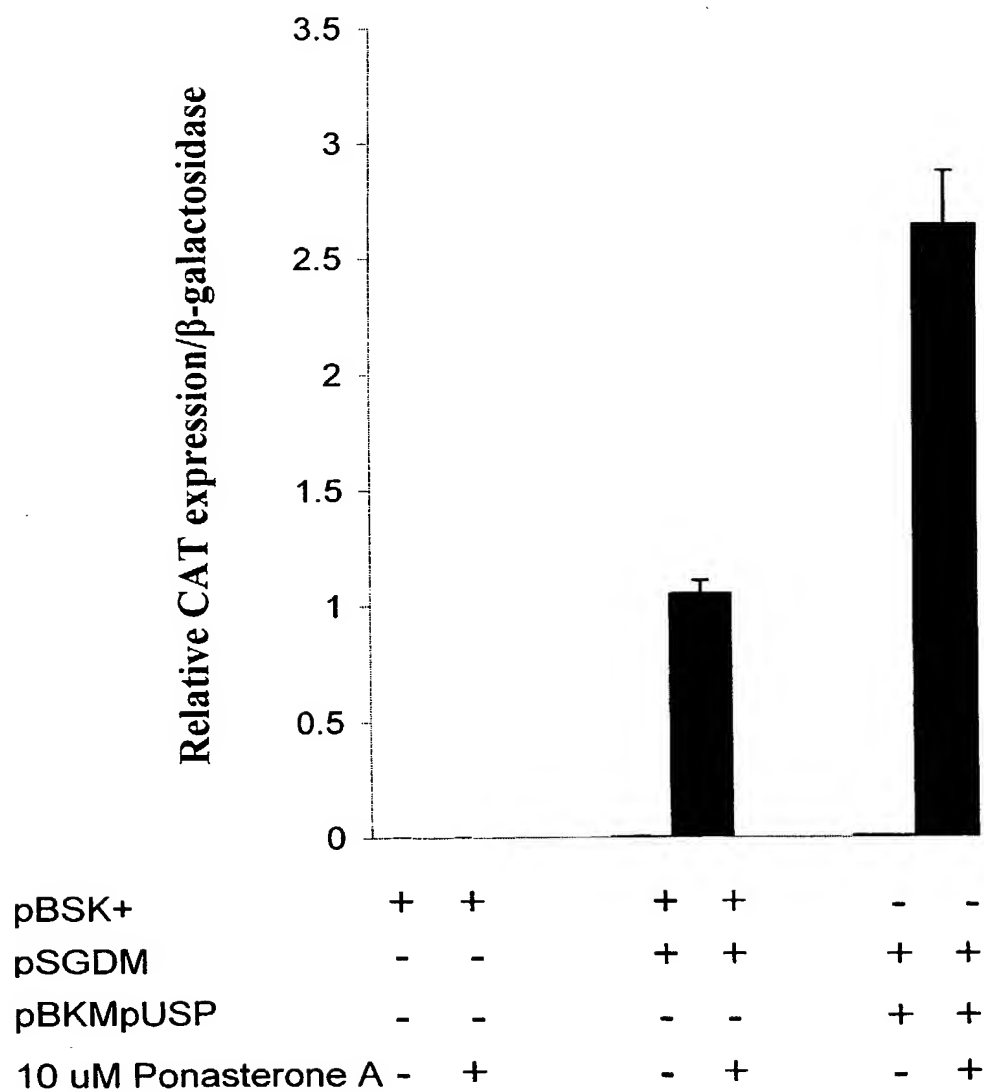


FIGURE 6

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**FIGURE 7**

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**FIGURE 8**

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Attorney Docket No. 53-99A

**JOINT INVENTORS' DECLARATION FOR PATENT APPLICATION  
AND POWER OF ATTORNEY**

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenship are as stated below our names.

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**Genetic sequences encoding steroid and juvenile hormone receptor polypeptides  
and uses therefor**

the specification of which was filed on 30 June 2000 as PCT/AU00/00799 and  
amended on 14 May 2001 ✓

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

**Prior Foreign Application(s)**

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application to which priority is claimed:

Country	Application No.	Date of Filing (day,month,year)	Date of Issue (day,month,year)	Priority Claimed 35 U.S.C.119
				Yes__ No__

10030729, 062002

**Prior Provisional Application(s)**

We hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial  
Number

Date of Filing  
(day, month, year)

**Prior U.S. Application(s) and PCT International Application(s)  
Designating the United States**

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT International application(s) designating the United States listed below:

Application Serial  
Number

Date of Filing  
(day, month, year)

Status(Patented, Pending, Abandoned)

09/346470

1 July 1999

Pending

Insofar as the subject matter of each of the claims in this application is not disclosed in the prior United States, foreign or PCT International application(s) to which priority has been claimed above in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

We hereby appoint, both jointly and severally, as our attorneys and agents with full power of substitution and revocation, to prosecute this application and any corresponding application filed in the Patent Cooperation Treaty Receiving Office, and to transact all business in the Patent and Trademark Office connected herewith the following attorneys and agents, their registration numbers being listed after their names:

9-  
Lorance L. Greenlee, Reg. No. 27,894; Ellen P. Winner, Reg. No. 28,547; Sally A. Sullivan, Reg. No. 32,064; Donna M. Ferber, Reg. No. 33,878; G. William VanCleave, Reg. No. 40,213; Susan K. Doughty, Reg. No. 43,595; Heeja Yoo-Warren, Reg. No. 45,495; Tamala R. Jonas, Reg. No. 47,688; and Mary Beth Vellequette, Reg. No. 47,903, all of Greenlee, Winner and Sullivan, P.C., 5370 Manhattan Circle, Suite 201, Boulder, CO 80303.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



1-00 (1) Full Name of First Inventor:

Residence:

Citizenship:

Post Office Address:

Ronald Johnston HILL

Forestville, NSW, Australia

Australian

Altona Avenue

Forestville

New South Wales 2087

Australia

AUX

(1) Signature

Ronald Hill

Date

18/4/02

2-00 (2) Full Name of Second Inventor:

Residence:

Citizenship:

Post Office Address:

Garry Noel HANNAN

Hunters Hill, NSW, Australia

Australian

32 Earl Street

Hunters Hill

New South Wales 2110

Australia

AUX

(2) Signature

[Signature]

Date

18/4/02